STRUCTURAL FEATURES OF THE PHOSPHORIBOSYL-TRANSFERASES AND THEIR RELATIONSHIP TO THE HUMAN DEFICIENCY DISORDERS OF PURINE AND PYRIMIDINE METABOLISM

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I. INTRODUCTION

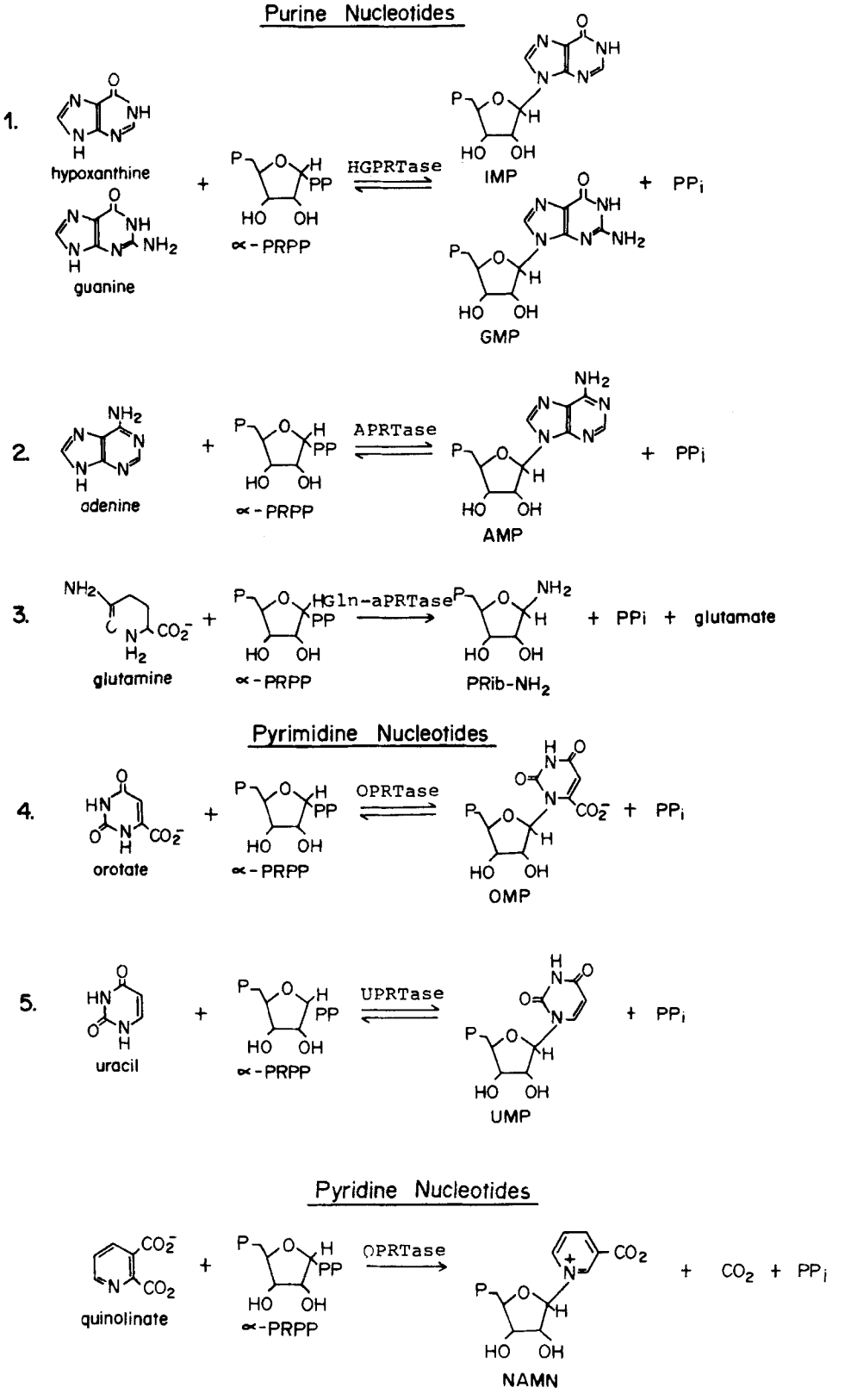
In most organisms, the biosynthesis of the purine, pyrimidine, and pyridine nucleotides, as well as the aromatic amino acids, histidine, and tryptophan, involves a group of ten enzymes known as phosphoribosyltransferases (PRTases). Each of these enzymes is highly specific for a nitrogenous, generally aromatic base, a divalent metal ion and α-D-5-phosphoribosyl 1-pyrophosphate (PRPP). In all cases, cleavage of the pyrophosphate moiety of PRPP is accompanied by the anomeric inversion of the ribofuranose ring resulting in a β-N riboside monophosphate (Figure 1). The participation of nonheme iron as well as nucleotide substrates or effectors has also been observed.

Several PRTases are allosterically regulated by a variety of effectors and hence function as key control points in their respective metabolic pathways. Generally these enzymes are multimeric assemblages composed of two to six subunits which sometimes occur as bifunctional complexes with a subsequent enzyme of the pathway. In some cases, the state of subunit aggregation can also influence the catalytic characteristics of the protein. Distinct isoenzymic species of the PRTases are not believed to exist although physical and kinetic variations occur between the PRTases of different organisms. In vertebrates, several PRTases exhibit a striking organ specificity while others are found in varying levels in most tissues. In all organisms, the PRTases are subcellularly confined to the soluble cytoplasmic fractions.

To date, seven of these proteins, hypoxanthine-guanine PRTase (HGPRTase), orotate PRTase (OPRTase), adenine PRTase (APRTase), quinolinate PRTase (QPRTase), ATP-PRTase, glutamine-amido PRTase (Gln-aPRTase), and anthranillate PRTase (Anth-PRTase) have been homogeneously isolated. Amino acid sequencing studies have been initiated only on QPRTase, ATP-PRTase, and Anth-PRTase. Despite the lesser degree of purity of the remaining PRTases, all have been physically and kinetically characterized. A detailed examination of each enzyme is the subject of the following sections.

II. PYRIDINE NUCLEOTIDE PHOSPHORIBOSYLTRANSFERASES

The biosynthesis of NAD from nicotinate was first observed in mammalian liver. The enzyme responsible, nicotinate PRTase (NPRTase), was subsequently isolated and found to require PRPP and magnesium as co-substrates. Later investigations



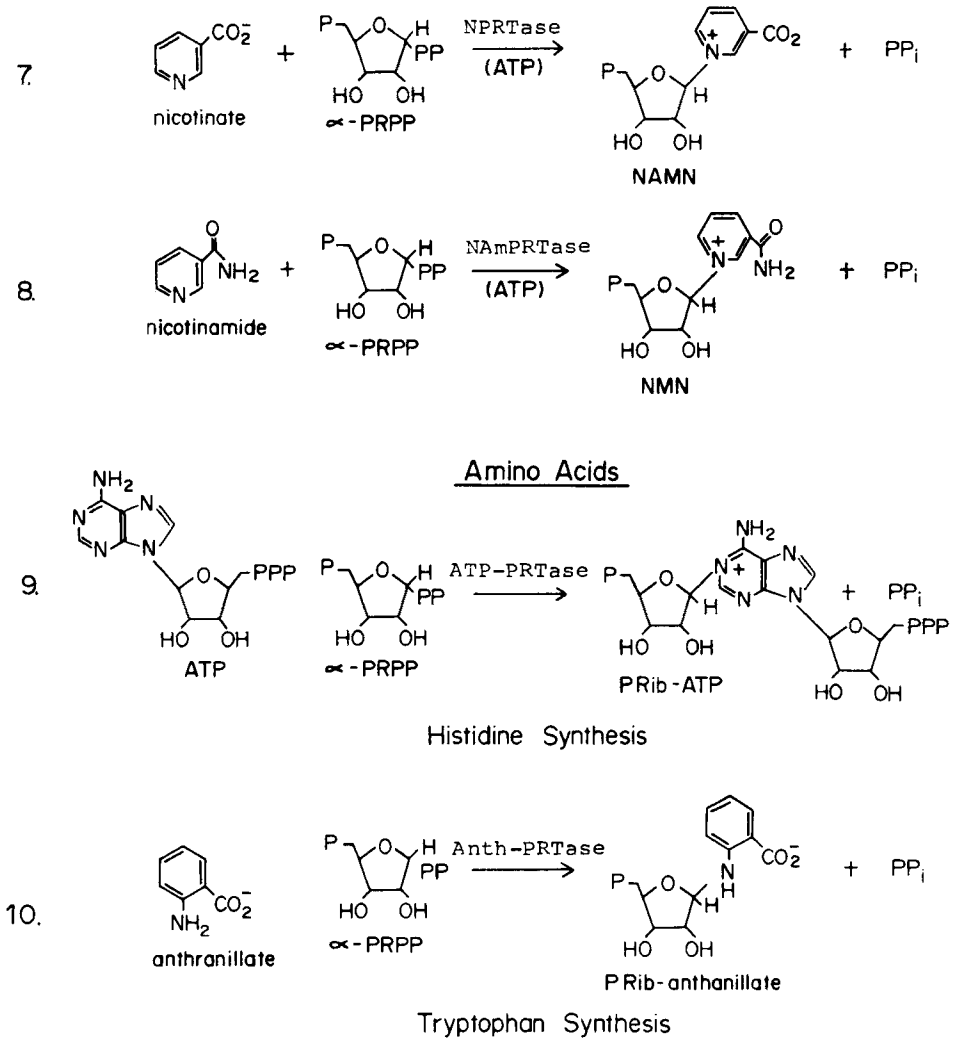


FIGURE 1. Reactions catalyzed by the phosphoribosyltransferases.

demonstrated that nicotinamide and quinolinic acid also served as precursors to NAD in similar divalent metal-PRPP requiring reactions. While quinolinic acid is synthesized in all organisms by one or more pathways, nicotinic acid and nicotinamide must be obtained exogenously. Consequently, biosynthesis of NAD from quinolinate via QPRTase is considered a *de novo* pathway while NPRTase and nicotinamide PRTase (NAmPRTase) are designated as salvage enzymes. The relationship of each enzyme to NAD synthesis is shown in Figure 2.

A. Nicotinate Phosphoribosyltransferase (NPRTase)

NPRTase (EC 2.4.2.11; Figure 1, #7) has been isolated in various states of purity from baker's yeast,[1-4] Ehrlich ascites cells,[5] *Astasia longa,*[6] bovine liver,[7] human erythrocytes,[8,9] *Bacillus subtilis,*[10] and several other microorganisms.[11-13] The enzymes from these organisms have been divided into three classes depending on their response to ATP. While the enzyme from the protozoan *A. longa* is not affected by ATP, a second class, represented by baker's yeast and microorganisms, is dependent on ATP for

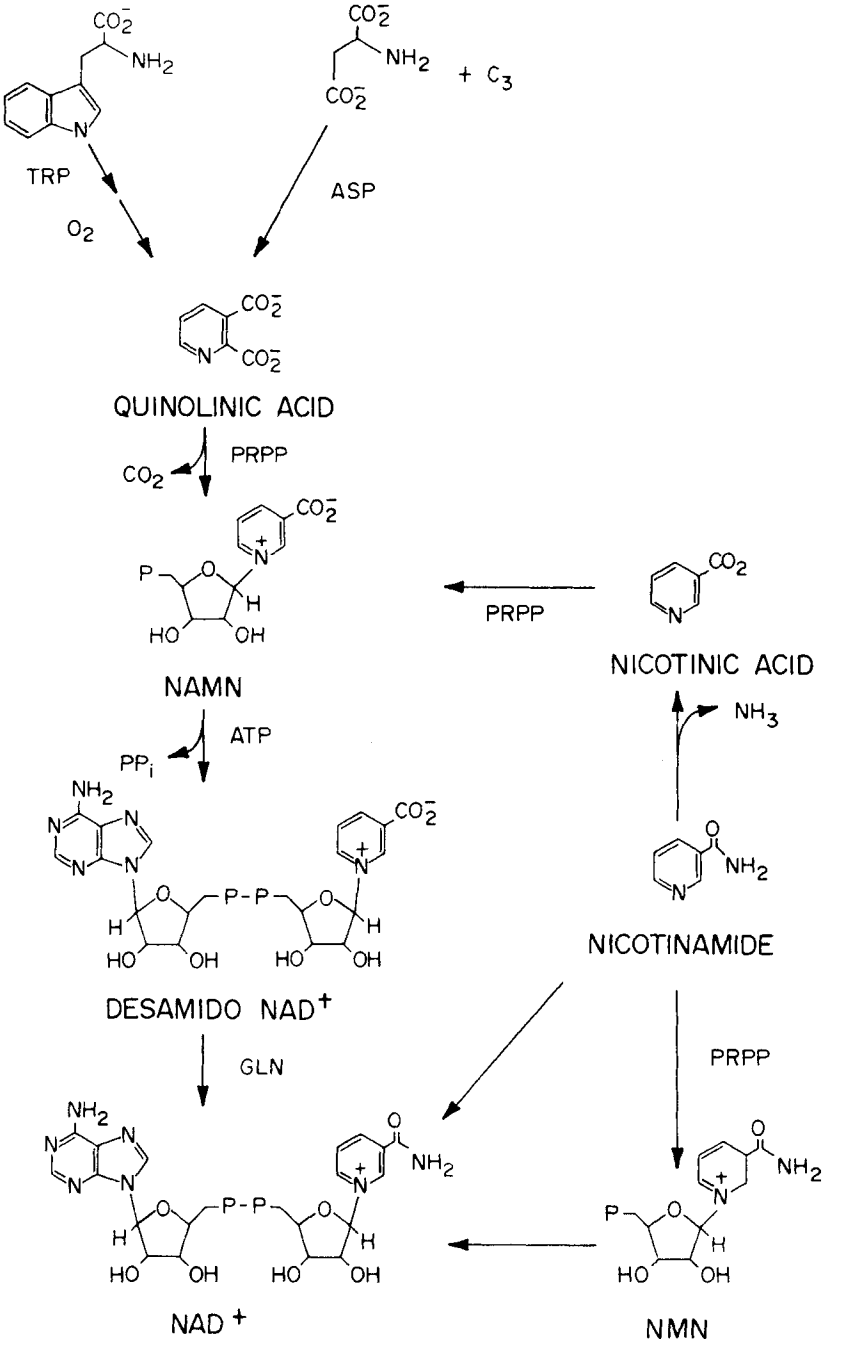


FIGURE 2. Pyridine nucleotide biosynthetic pathways.

activity. In the latter cases, ATP acts as a co-substrate and is cleaved to ADP and Pi stoichiometrically with nicotinate mononucleotide formation.[2,3] The third group, found in mammals, also stoichiometrically cleaves ATP although it is not necessary for activity.[7-9] In this case ATP acts as a positive allosteric effector by lowering the Michaelis constants for the substrates.[7-9] A reduction in K_ms from 20- to 50-fold has been reported

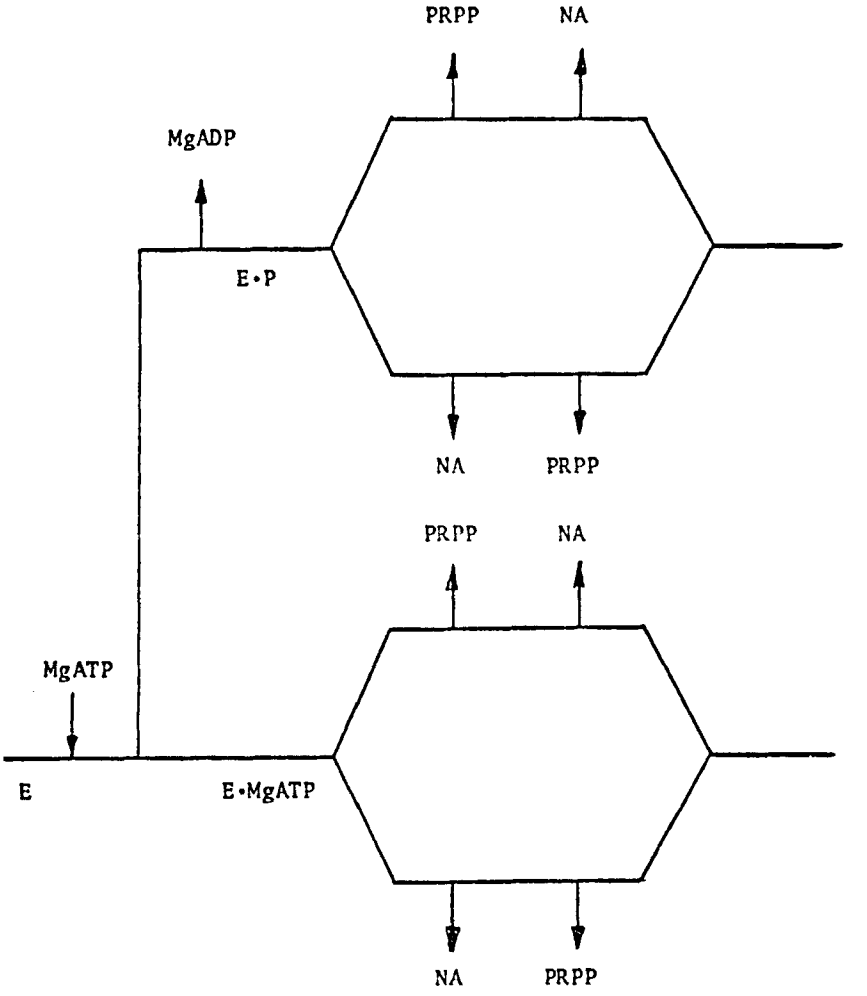


FIGURE 3. Proposed mechanism of yeast nicotinate phosphoribosyltransferase.

for the human erythrocyte enzyme for PRPP and nicotinate, respectively.[9] Studies with bovine liver NPRTase indicate a single ATP binding site.[7]

Detailed kinetic studies on the mechanism of NPRTase and the involvement of ATP have been done only on the baker's yeast system.[4] In the absence of PRPP and nicotinate, ATP will phosphorylate the enzyme via a Ping-Pong® type mechanism after which substrate addition is random sequential. Release of inorganic phosphate occurs only after addition of both remaining substrates. If, however, all substrates are present simultaneously, a sequential mechanism operates where ATP is bound first followed by random binding of nicotinate and PRPP. This mechanism is illustrated in Figure 3. Turnover numbers varying from 4 to 99 molecules NAMN per minute per enzyme molecule have been observed.[3,4,9]

NPRTase is highly specific for nicotinic acid showing no activity toward nicotinamide, quinolinate, purines, or pyrimidines.[2] However, the purine nucleotides GTP and ITP and, to a lesser extent, the pyrimidines CTP and UTP may replace the ATP requirement.[3,7,14] In all cases, the nucleotide triphosphate interacts with the enzyme as a Mg^{++} complex.[3,4] The stability of the yeast protein to thermal denaturation is enhanced in

Table 1a
PHYSICAL AND KINETIC PROPERTIES OF THE PHOSPHORIBOSYLTRANSFERASES

Enzyme	Native mol wt	Number of subunits	Sedimentation coefficient $s^0_{20,w}$
NPRTase	43,000 (3)	1 (3)	—
	73,000—82,000 (7)	2 (3, 9)	
	86,000 (9)		
NAmPRTase	64,000 (16)	—	—
QPRTase	70,000 (28)	2 (28)	6.8 (31)
	165,000 (25)	3, 2 species (25)	7.8 (26)
	168,000 (40)	6 (37, 40)	8.0 (27)
	210,000 (37)	8 (37)	9.9 (25)
	222,000 (26)		
Gln-aPRTase	194,000—224,000 (46)	Min. 2 (51, 52)	9.3 (57)
	198,000—205,000 (56)	3—4 (46)	10.0 (47, 51, 52)
	200,000 (45, 47)	4 (45, 47, 57)	
	202,000—210,000 (57)		
	270,000 (51, 52)		
HGPRTase	57,000 (64)	4 (79, 85, 86)	5.0 (69)
	98,000 (86)		5.5—5.6 (77)
	105,000—110,000 (85)		5.78 (79)
	108,000 (79)		5.9 (78)
			6.65 (85)
APRTase	22,000 (90, 93)	1 (90, 93)	2.85 (90, 93)
	34,000 (89, 92)	2 (91)	3.32 (91)
	38,200 (91)		3.35 (89, 92)
	40,000 (94)		
	45,000 (100)		
OPRTase	26,000 (105, 113)	2 (105, 108, 113)	2.9 (106)
	32,000 (106)		3.4 (103)
	39,000 (103)		
	40,000 (108)		
UPRTase	80,000 (121)	2 Distinct polypeptide species (121)	—
	100,000 (122)	Subunit structure of unknown composition (119)	
ATP-PRTase	67,000 (134)	2 (134)	8.83 (129)
	200,000 (142)	6 (129)	9.6 (126)
	215,000 (129)		
Anth-PRTase	45,000 (145)	1 (145)	4.1 (153)
	80,000 (146)	2 (146—149)	4.4 (157)
	130,000 (147—149)	6 (141)	5.8 (148)
	200,000 (141)		

the presence of some nucleotide analogs as well as the substrates PRPP and nicotinate.[2,3] Regardless of the class, the enzyme has a broad pH optimum ranging from 5 to 10 with a maximum near 8.0.[2]

The molecular weight of the native enzyme from mammals has been estimated to be between 43,000 and 86,000, with the higher values obtained in the presence of ATP.[3,7,9] The yeast enzyme has a molecular weight of approximately 43,000, suggesting the mammalian enzyme is a dimer.[3,9] A summary of the properties of the enzyme is given in Tables 1a, 1b, 1c, and 1d.

Table 1b
PHYSICAL AND KINETIC PROPERTIES OF PHOSPHORIBOSYLTRANSFERASES

Enzyme	Frictional ratio (f/f_0)	Stokes radius (Å)	Isoelectric point (PI)	pH optimum
NPRTase	—	—	—	6.5—8.0 (9)
				7.5 (7)
				8.0 (3, 4)
				8.0—9.0 (2)
NAmPRTase	—	—	—	6.5—9.0 (18)
				7.5—9.5 (15)
				8.0 (16)
				8.5—9.0 (17)
QPRTase	1.08 (25)	49.1 (27)	4.3 (27)	6.1 (36)
	1.33 (27)	57.8 (37)	5.3 (26)	6.2 (30)
	1.49 (37)		6.0 (28)	6.5—7.7 (28)
				7.0 (31)
				7.1 (25)
				8.5—9.0 (26)
Gln-aPRTase	1.54 (51)	65.7 (51)	5.2 (57)	6.0—9.0 (57)
				6.5—8.5 (56)
				6.6—8.6 (46)
				6.8—7.4 (49)
				7.5 (50)
HGPRTase	—	36.0 (60, 69)	5.1 (64)	7.1—9.1 (59)
		36.6 (79)	5.6, 5.7, 5.9 (78)	8.4 (65)
			5.6, 5.85, 5.9 (70)	8.5 (64)
			5.7, 5.8, 6.0 (60)	10.5 (61)
			6.2, 6.4, 6.6 (61)	
APRTase	1.16 (89, 91)	24.9 (89, 92)	4.78 (89)	7.4—9.5 (89, 92)
		26.0 (91)	4.85 (92)	7.8 (94)
			5.1 (95)	8.6 (95)
			5.65 (90, 93)	10.0 (90, 93)
OPRTase	—	—	—	6.9, 8.0—8.5 (101)
				8.0 (102, 109)
				8.0—9.2 (105)
				8.5—9.0 (103)
UPRTase	—	—	5.27, 5.35 (121)	7.8 (118)
				7.8—8.0 (120)
				8.5 (122)
ATP-PRTase	—	—	—	8.5 (128)
Anth-PRTase	—	—	—	7.5 (147)
				7.6 (148)

B. Nicotinamide Phosphoribosyltransferase (NAmPRTase)

In addition to NPRTase, a similar enzyme utilizing nicotinamide, Mg^{++}, PRPP, and ATP, NAmPRTase (EC 2.4.2.12; Figure 1, #8), has been partially purified from rat liver,[15,16] erythrocytes,[17] and Ehrlich ascites cells.[18] Again, two classes of enzyme appear to exist based on the effect of ATP. The ATP requirement for the rat liver enzyme can be replaced with elevated levels of PRPP-Mg^{++}[16] while ATP is an absolute requirement in the other systems. Studies with the Ehrlich ascites protein indicate that other nucleotides including GTP, UTP, CTP or their mono- and diphosphates will not substitute for ATP.[18]

Kinetic studies on the rat liver enzyme have shown ATP to be a positive allosteric effector. The reaction mechanism appears to be ordered or iso-Theorell-Chance with

Table 1c
PHYSICAL AND KINETIC PROPERTIES OF PHOSPHORIBOSYLTRANFERASES

Enzyme	Mechanism	Michaelis constants (M)	
		Preferred base	PRPP
NPRTase	Hybrid sequential and hybrid Ping-Pong® (4)	1.85×10^{-6} (2) 2.5×10^{-6} — 2.1×10^{-5} (7) 2.4×10^{-5} (8)	7.7×10^{-6} (2) 4.0×10^{-5} — 5.0×10^{-4} (7)
NAmPRTase	Ordered sequential or iso-Theorell-Chance (16)	1.0×10^{-7} (17) 2.96×10^{-6} (15)	3.8×10^{-6} (17) 1.62×10^{-5} (18) 3.57×10^{-5} (15)
QPRTase	Ordered sequential (38) Sequential (25, 26, 28)	7.0×10^{-6} (31) 1.2×10^{-5} (28) 6.0×10^{-5} (30) 1.2×10^{-4} (25, 36) 1.3×10^{-4} (26)	4.5×10^{-5} (28) 5.0×10^{-5} (30) 7.4×10^{-5} (25) 1.1×10^{-4} (26) 1.8×10^{-4} (36)
Gln-aPRTase	Ordered sequential (57)	1.0×10^{-3} (48, 52) 1.1×10^{-3} (57) 1.4×10^{-3} (56) 1.6×10^{-3} (49, 50) 1.7×10^{-3} (46) 2.0×10^{-3} (53)	6.0×10^{-5} (48) $3.0—9.0 \times 10^{-4}$ (53) 4.8×10^{-4} (50)
HGPRTase	Hybrid ordered sequential Ping-Pong® (75) Ordered sequential (74) Ordered sequential forward, random sequential reverse (80)	5.2×10^{-7} (61) 2.3×10^{-5} (64) 2.5×10^{-5} (75) 1.2×10^{-4} (65) 2.4×10^{-4} (74)	4.0×10^{-6} (74) 5.3×10^{-6} (61) 5.0×10^{-5} (64) 2.0×10^{-4} (65) 2.6×10^{-4} (68) 2.0×10^{-3} (75)
APRTase	Ping-pong® (89, 94, 96—98)	1.0×10^{-6} (90, 93) 1.25×10^{-4} (94) 1.4×10^{-4} (92, 96)	5.0×10^{-6} (90, 93) 6.0×10^{-6} (92) 2.0×10^{-5} (94) 3.7×10^{-5} (99) 6.2×10^{-5} (96)
OPRTase	Ping-Pong® (108)	1.3×10^{-6} (104) 1.6×10^{-6} (102) 2.0×10^{-6} (110) 5.5×10^{-6} (106) 1.7×10^{-5} (109) 2.2×10^{-5} (105) 3.2×10^{-5} (101) 3.3×10^{-5} (103) 3.8×10^{-5} (108)	4.1×10^{-6} (104) 4.5×10^{-6} (102) 1.5×10^{-5} (105) 1.6×10^{-5} (110) 2.1×10^{-5} (101) 3.2×10^{-5} (106) 3.5×10^{-5} (108) 6.2×10^{-5} (103)
UPRTase	Ping-Pong® (121)	7.0×10^{-7} (122) 2.0×10^{-6} (119) 4.0×10^{-6} (117) 7.7×10^{-6} (118) 8.5×10^{-6} (120) 2.1×10^{-5} (121)	1.1×10^{-5} (122) 2.0×10^{-5} (117, 120) 2.6×10^{-5} (121)
ATP-PRTase	Ordered sequential (123, 124)	1.1×10^{-4} (123) 2.0×10^{-4} (126) 3.4×10^{-4} (127)	1.1×10^{-5} (123) 5.2×10^{-5} (127) 6.7×10^{-5} (126)
Anth-PRTase	Ordered sequential (147)	4.0×10^{-6} (148) 6.7×10^{-6} (147)	8.3×10^{-6} (147) 1.0×10^{-5} (148) 1.6×10^{-5} (154)

Table 1d
PHYSICAL AND KINETIC PROPERTIES OF THE PHOSPHORIBOSYLTRANSFERASES

Enzyme	Turnover number (mol prod per minute per mole enzyme)	Prosthetic groups or additional substrates	Substrate-induced thermal stability	Essential residues	Exogenous sulfhydryl requirement
NPRTase	4 (4) 99 (3)	ATP (2—4, 7, 9, 11)	ATP (3) GTP (3) Nicotinate (3) Pi (2, 3) PRPP (2, 3)	—	—
NAmPRTase	<1 (15)	ATP (15—18)	—	—	—
QPRTase	9.5 (27) 54 (28) 145 (24)	—	Quinolinate (28)	Lys, His, Arg, Cys (39)	+(25, 28) −(39)
Gln-aPRTase	27 (48) 508 (57) 3595 (46)	Fe_3S_2 (45) Nonheme iron (48, 57)	—	Cys (45, 46, 48)	+(45, 46, 48)
HGPRTase	125 (60) 240 (69) 734 (61) 1435 (78)	—	PRPP (61, 71, 76)	Cys (71, 75) Lys (71, 81—83)	+(65, 71, 75)
APRTase	22 (90) 325 (89) 560 (94) 832 (91)	—	AMP, adenine, PPi (96, 98) $(NH_4)_2SO_4$ (95) PRPP (96—98)	Cys (90, 92, 96, 97 Lys (97)	+(89, 90, 96, 97) −(94, 99)
OPRTase	1320 (116) 2600 (108)	—	—	—	+(102, 104, 105, 110, 113) −(103)
UPRTase	40 (121)	—	UMP (121)	Cys (121)	+(117)
ATP-PRTase	862, (129) 1148 (127)	—	His (126)	Cys (126, 130, 143) His (126)	+(126, 129, 130)
Anth-PRTase	85 (147) 186 (145) 754 (148) 1641 (146)	—	—	Cys (153) Lys (156)	+(145, 147—149)

PRPP bound first and PPi the first product released.[16] Michaelis constants for the substrates vary over several orders of magnitude depending on the source of the enzyme (Table 1c). The turnover number for the rat liver enzyme has been estimated to be less than 1 molecule of NMN produced per hour per enzyme molecule.[15] Similar to NPRTase, NAmPRTase is active over a broad pH range with a maximum near 8.0.[15,17,18] However unlike the nicotinate enzyme, NAmPRTase, is rapidly inactivated at an acid pH[15,18] and is moderately thermally stable.[15,16]

The molecular weight of the rat liver enzyme has been estimated to be about 64,000[16] using gel filtration. No data are available on the subunit composition of NAmPRTase.

C. Quinolinate Phosphoribosyltransferase (QPRTase)

In the absence of exogenous nicotinate or nicotinamide, most organisms are still able to synthesize NAD.[19-23] The enzyme responsible, QPRTase (EC 2.4.2.19; Figure #6), has been homogeneously isolated or crystallized from bacteria,[24-26] hog liver,[27] castor

bean,[28] "Shiitake" mushroom,[29] and beef liver.[30] In mammals, QPRTase is found only in liver and kidney, and consequently these organs are the only sites of *de novo* NAD synthesis.[31] Quinolinate and Mg^{++}-PRPP are the sole reactants[25,28,31,32] in all organisms although the synthesis of quinolinate varies between plant, mammalian, and bacterial systems.[20-23,33-35]

Similar to NPRTase and NAmPRTase, QPRTase is highly specific for its nitrogenous substrate, quinolinic acid (QA). Studies with substrate analogs have shown that positions number two- and three-carboxylate groups are both necessary for binding.[28,32] The metal ion requirement, however, can be substituted by other divalent cations such as Co^{++}, Zn^{++}, Mn^{++}, and Cd^{++}.[26,28,30,36] While a divalent metal ion is necessary for catalysis, beyond an optimal concentration it becomes inhibitory.[30,36,37] This effect may be reversed by the addition of purine or pyrimidine nucleoside triphosphates which presumably act by the chelation of metal ion. Consequently, at optimal metal ion concentrations, nucleoside triphosphates are inhibitory.[32]

Kinetic studies on the bacterial, plant, and mammalian enzymes have shown that the substrates bind via a sequential mechanism forming a ternary complex before any product is released.[25,28,38] The order of substrate binding has not been established although preliminary work in this laboratory suggests the order to be: (1) PRPP ↓, (2) QA ↓, (3) NAMN ↑, (4) PPi ↑. The reaction products of QPRTase have been identified as only NAMN, inorganic pyrophosphate, and CO_2 in all organisms. The elimination of the two-carboxy group has been postulated to be nonenzymic due to the enhanced electro-negativity of the ring carbon number 2 on formation of the quaternary ring ribonucleotide.[25] Michaelis constants for both substrates are similar in magnitude to those from NPRTase and NAmPRTase although a considerable variation occurs among species (Table 1c). Like NPRTase, the thermal stability of QPRTase is enhanced by the presence of its substrates.[28]

Chemical modifications of the hog liver enzyme have demonstrated that Arg, Lys, Cys, and His residues are necessary for activity, with Lys, Cys, and His associated with the QA binding site.[39] The enzyme contains five titratable SH groups per subunit and the single exposed residue appears to be near the active center.[39] While QPRTase from mammalian tissues does not require added thiol protectants, the bacterial and plant enzymes require sulfhydryl reagents for stability.[25,28] The amino acid composition of hog liver QPRTase shows a higher-than-average content of hydrophobic amino acids and a greater ratio of acidic to basic residues, resulting in the acidic isoelectric point of 4.32 (Tables 1b and 2). The amino terminus of the enzyme appears to be blocked.[40]

The entire amino acid sequence of hog liver QPRTase has not yet been determined although some sequence data are available on several isolated peptide fragments.[216] These fragments do not show any sequence homologies with the other PRTases where sequence information is known. However, an active center lysyl peptide has recently been identified by convalently labeling the hog liver protein with the radioactive product analog [6-^{14}C] nicotinic acid mononucleotide 2',3'-dialdehyde.[216] The first nine residues of this fragment (Arg, Gly, Val, Ser, Phe, Lys, Ser, Cys, Leu) contain the labeled lysine and were found to be identical to residues 143 to 150 of ATP-PRTase from *Salmonella typhimurium*.[132]

QPRTase from hog liver contains six identical subunits of molecular weight 28,000 to 34,000.[37,41] The molecular weight of the castor bean enzyme subunit is similar at 35,000 although the native molecule appears to exist as a dimer. Furthermore, it has been observed that more than one polypeptide species may be present.[28] The pseudomonad protein has the same parent molecular weight as the mammalian enzyme, but seems to be composed of three identical subunits of molecular weight 54,000[25] (Table 1a). However, QPRTase from the bacterium *Alcaligenes eutrophus* has been reported to contain eight

Table 2
AMINO ACID COMPOSITION OF 5 PHOSPHORIBOSYLTRANSFERASES

	QPRTase	Gln-aPRTase		HGPRTase	APRTase	ATP-PRTase	PRTase average ± SD	Average protein composition (%)[215]
		E. coli	Pigeon liver					
Glx	25 (9.9)	54 (10.4)	34 (7.5)	16 (7.0)	21 (11.4)	42 (12.8)	9.8 ± 2.2	9.5
Asx	16 (6.3)	66 (12.7)	41 (9.0)	31 (13.5)	11 (5.9)	30 (9.1)	9.4 ± 3.2	9.7
Lys	8 (3.2)	18 (3.5)	28 (6.2)	17 (7.4)	9 (4.9)	11 (3.3)	4.8 ± 1.7	6.6
His	5 (2.0)	10 (1.9)	10 (2.2)	5 (2.2)	2 (1.1)	4 (1.2)	1.8 ± 0.5	2.1
Arg	9 (3.6)	39 (7.3)	25 (5.5)	12 (5.2)	13 (7.0)	23 (7.0)	5.9 ± 1.4	4.5
Thr	13 (5.1)	20 (3.9)	24 (5.3)	12 (5.2)	6 (3.2)	13 (4.0)	4.5 ± 0.9	5.8
Ser	14 (5.5)	19 (3.7)	25 (5.5)	14 (6.1)	11 (5.9)	17 (5.2)	5.3 ± 0.9	7.2
Pro	16 (6.3)	21 (4.1)	24 (5.3)	14 (6.1)	12 (6.5)	14 (4.3)	5.4 ± 1.0	5.6
Gly	25 (9.9)	38 (7.3)	40 (8.8)	19 (8.3)	19 (10.3)	24 (7.3)	8.7 ± 1.3	8.8
Ala	40 (15.8)	49 (9.5)	41 (9.0)	12 (5.2)	17 (9.2)	29 (8.8)	9.6 ± 3.4	8.7
Cys	5 (2.0)	5 (0.9)	18 (4.0)	5 (2.2)	3 (1.6)	5 (1.5)	2.0 ± 1.1	3.1
Val	26 (10.3)	36 (6.9)	33 (7.3)	18 (7.8)	14 (7.6)	20 (6.1)	7.7 ± 1.4	6.6
Met	3 (1.2)	4 (2.1)	9 (2.0)	5 (2.2)	1 (0.5)	17 (5.2)	2.2 ± 1.6	1.7
Ile	3 (1.2)	43 (8.3)	20 (4.4)	11 (4.8)	7 (3.8)	24 (7.3)	5.0 ± 2.5	4.6
Leu	31 (12.3)	40 (7.7)	49 (10.8)	20 (8.7)	32 (17.3)	46 (14.0)	11.8 ± 3.5	7.2
Tyr	3 (1.2)	17 (3.3)	13 (2.9)	10 (4.3)	5 (2.7)	6 (1.8)	2.7 ± 1.1	3.5
Phe	9 (3.6)	23 (4.4)	14 (3.1)	9 (3.9)	9 (4.9)	4 (1.2)	3.5 ± 1.3	3.5
Trp	2 (0.8)	—	6 (1.3)	0 (0)	—	—	0.7 ± 0.6	1.2
Σ Glx, Asx	41 (16.2)	120 (23.2)	75 (16.5)	47 (20.4)	32 (17.3)	72 (21.9)	19.3 ± 3.0	19.2
Σ His, Arg, Lys	22 (8.7)	67 (12.9)	63 (13.9)	34 (14.8)	24 (13.0)	38 (11.6)	12.5 ± 2.1	13.2
Σ Tyr, Trp, Phe	14 (5.5)	40[a] (7.7)	33 (7.3)	19 (8.3)	14[a] (7.6)	10[a] (3.0)	7.0 ± 1.4	8.2
Σ Leu, Val, Ile, Phe, Met	72 (28.5)	153 (29.5)	125 (27.5)	63 (27.4)	63 (34.1)	111 (33.7)	30.1 ± 3.0	23.6
Total number residues	253[40]	518[a,46]	454[48]	230[78]	185[a,91]	329[a,130]		

Note: Bracketed quantities represent percentage of total residues.

[a] Excluding tryptophan.

subunits of molecular weight 27,500.[26] Frictional ratios obtained from crystalline bacterial and mammalian QPRTase range from 1.08 to 1.49 indicating that both native proteins are compact despite possible differences in subunit composition.[25,27] Recent studies with the hog liver enzyme have suggested that QPRTase is a glycoprotein,[37] although the observation could not be confirmed in this laboratory.

III. PURINE NUCLEOTIDE PHOSPHORIBOSYLTRANSFERASES

The initial and rate-limiting step of purine nucleotide biosynthesis is catalyzed by the regulatory enzyme glutamine-amido PRTase (Gln-aPRTase). The product, 5-phosphoribosylamine (see Figure 1), is then transformed to inosine monophosphate (IMP) through a series of nine complex enzymatic steps utilizing four molecules of ATP. The nucleoside monophosphates of adenine, guanine, and xanthine are subsequently formed by modifying IMP (Figure 4).

In addition to *de novo* synthesis, purine nucleotides are also derived from preformed purine bases. These bases, which may arise from the degradation of nucleic acids or in the diet, are reutilized by the salvaging enzymes HGPRTase and APRTase. Analogous to the PRTases of pyridine nucleotide metabolism, Gln-aPRTase, HGPRTase, and APRTase utilize α-PRPP, a divalent metal ion (ideally Mg^{++}) and a nitrogenous base. However, base specificity for the purine PRTases is somewhat broader. The scheme of purine nucleotide synthesis and reutilization is shown in Figure 4.

A. Glutamine-amido Phosphoribosyltransferase (Gln-aPRTase)

Gln-aPRTase (EC 2.4.2.14; Fig. 1, #3) has been characterized in bacteria,[42-46] yeast,[47] pigeon liver,[48] human lymphoblasts,[49] human placenta,[50-54] and other mammalian systems.[49,50,55,56] The enzyme has been isolated to homogeneity from pigeon and chicken liver,[48,57] *B. subtilis*,[45] *Escherichia coli*,[46] and rat liver.[56] As is characteristic of most PRTases, Gln-aPRTase is highly specific for α-PRPP.[50] Magnesium or manganese is necessary for optimal catalytic activity, although Co^{++} and Ca^{++} are also somewhat effective.[49,50] The nitrogenous ligand in this case is either glutamine or ammonia.[46,50,53] The binding sites for each alternate substrate are topologically separate although site-site interaction has been observed in the human placental[53] and *E. coli*[46] enzymes. In addition to substrate binding sites Gln-aPRTase exhibits one or more type of inhibitory nucleotide binding sites.[48-50,53,57] These feedback regulatory sites have the highest affinity for the purine nucleotides AMP and GMP with less activity toward their di- and triphosphates or pyrimidine nucleotides.[46,48-50,53,56,57] The kinetics of purine nucleotide inhibition also vary with the source of the enzyme, from simple competitive inhibition for PRPP[49] to more complex allosteric interactions with both substrates.[46,50,56]

The synthesis of 5-phosphoribosylamine by Gln-aPRTase is irreversible and proceeds via a sequential mechanism with PRPP binding first.[52,53,57] The Michaelis constants for each substrate are somewhat higher than those of other PRTases, ranging from 0.06 m*M* to 2.0 m*M* (Table 1c). Gln-aPRTase exhibits a broad pH profile with a maximum near 7.0.[46,49,50,56,57] Turnover numbers at the pH optimum vary from 125 to 3595 in the pigeon liver[48] and *E. coli*[46] enzymes, respectively.

Gln-aPRTase from all species studied appears to be a tetrameric molecule with a subunit molecular weight of approximately 50,000[45,46,48,51,57] In vivo, monomers (PI = 5.2) may be covalently linked through disulfide bonds into functional dimers, two of which associate into a tetrameric species.[45,48,51,57] Furthermore, the equilibrium between dimers and tetramers is influenced by purine nucleotides as well as PRPP. Human placental Gln-aPRTase dimers associate to an inactive tetramer in the presence of purine nucleotides while the tetramer is converted to active dimers by PRPP.[51]

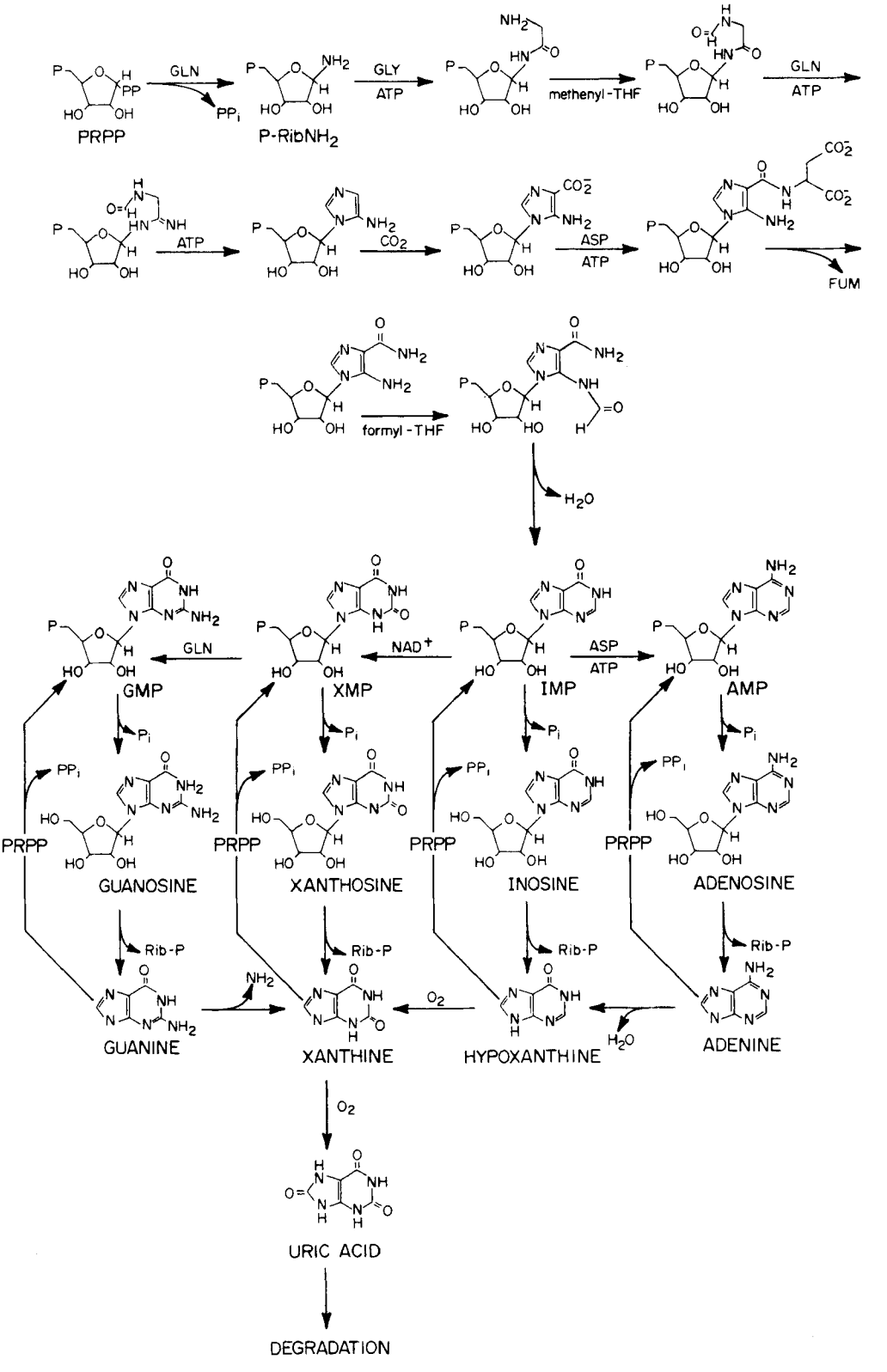


FIGURE 4. Purine nucleotide synthesis and reutilization.

However, effector-induced association is not observed with the avian liver enzyme and dimeric and tetrameric forms are both active.[48] The amino acid compositions of avian liver and *E. coli* Gln-aPRTase have recently been determined and are given in Table 2. As can be seen, the amino acid compositions of the two enzymes differ considerably.

A unique feature of Gln-aPRTase is the presence of three nonheme Fe^{++} atoms in each monomer.[45,48,57] The iron atoms are necessary for catalytic activity[45,46,48] and have been proposed to be NH_3-carrying centers.[46,58] A single iron atom associated with the PRPP binding site can be removed by chelators while the remaining two are released only in the presence of denaturing concentrations of thiols.[48] Gln-aPRTase from *B. subtilis* contains two atoms of inorganic sulfide per subunit and a single Fe^{++} center and a ferredoxin-like Fe_2S_2 center has been suggested.[45] However, the pigeon liver enzyme apparently does not contain acid labile sulfur and a monomer-S-Fe-S-monomer model has been proposed.[48] In contrast to Gln-aPRTase from *B. subtilis* and pigeon liver, the *E. coli* enzyme has been reported to contain no iron, and iron salts do not enhance the activity of this enzyme.[46]

B. Hypoxanthine-Guanine Phosphoribosyltransferase (HGPRTase)

HGPRTase (EC 2.4.2.8; Figure 1, #1) catalyzes the Mg^{++}-dependent transfer of a ribosyl phosphate group from α-PRPP to the number 9 imidazole nitrogen atom of the purine bases hypoxanthine, guanine and, to a lesser extent, xanthine.[59-61] The enzyme has been characterized in baker's yeast,[62-64] several bacteria,[65-67] rodent brain and liver,[61,68-71] HeLa cells,[72] mouse lymphoma,[73] and most extensively in human erythrocytes.[59,60,70,74-80] Magnesium or manganese are the most effective cations while other cations such as Ba^{++}, Ca^{++}, and Zn^{++} are inhibitory.[59,64,65,70] As with other PRTases, Mg^{++}-PRPP is the active form of PRPP[59,71,75,80] and beyond and optimal concentration Mg^{++} is inhibitory.[59,64,80] Although the enzyme is specific for PRPP, six thio or oxo derivatives as well as 1-methyl analogs are bound to the enzyme.[59] The imidazole portion of the purine ring is necessary for substrate binding although imidazole alone is not bound.[59]

The amino acid residues involved in binding the substrates have been studied by chemical modification of the rat liver and human erythrocyte enzymes.[71,75] HGPRTase was found to be inactivated by sulfhydryl and free-amino modifying reagents but protected from inactivation by Mg^{++}-PRPP.[71,75] The involvement of an amino group in binding PRPP has also been suggested by the irreversible inactivation of HGPRTase by periodate-oxidized PRPP and GMP.[81-83] Here the presumptive formation of a Schiff's base of a free-amino function and the ribosyl-dialdehyde is prevented by the inclusion of Mg^{++}-PRPP.[81-83]

Early kinetic studies on human HGPRTase established that PRPP was first bound to the enzyme.[74,75] However, depending on the concentration of magnesium ion, a Ping-Pong®-type mechanism involving a phosphoribosyl-enzyme or an ordered sequential mechanism appeared to be in operation.[75] More recently, an analysis of the reaction in both forward and reverse directions has suggested a hybrid mechanism, where the ordered addition of PRPP, then purine, is followed by the random release of Mg^{++}-nucleoside phosphate and Mg^{++}-PPi complexes[77] (Figure 5). The Michaelis constants for hypoxanthine, guanine and PRPP are fairly typical for PRTases varying from 10^{-4} to 10^{-6} *M* (Table 1c). The enzyme is active over a broad pH range with either guanine or hypoxanthine with an alkaline optimum of 8.0 to 10.0.[59,61,64,65,84] The turnover number for HGPRTase ranges from 125 to 1435 (Table 1d) depending on the source of the enzyme.

The molecular weight of native human erythrocyte HGPRTase has been variously estimated as 60,000 to 85,000[59,60,76-79,84,85] with similar values obtained for Chinese hamster, rat brain[61,70,84] and mouse liver.[69] A single subunit species with molecular

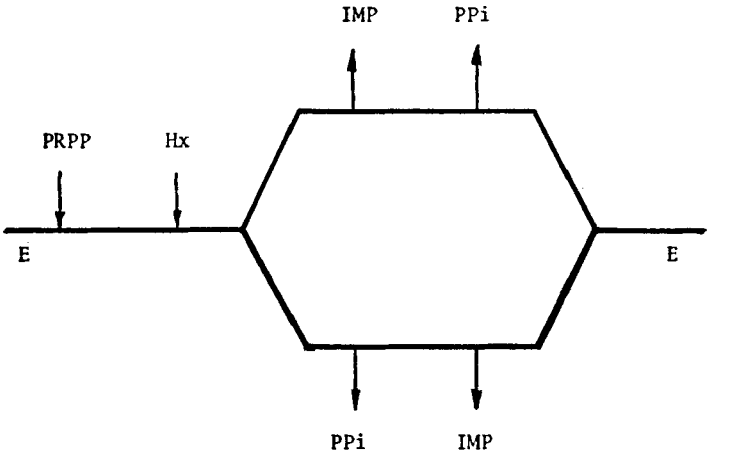


FIGURE 5. Proposed mechanism of human erythrocyte hypoxanthine-guanine phosphoribosyl-transferase.

weights from 25,000 to 35,000 have been consistently found, suggesting a dimeric or trimeric parent molecule.[61,69,70,76-79,84,85] However, cross-linking highly purified human HGPRTase (subunit mol wt 24,000) with imidoesters or glutaraldehyde clearly indicates a tetrameric structure.[79] Tetrameric and dimeric heteropolymeric species have also been observed in hybrid mouse-human cells.[86] Also, gel gradient electrophoresis of the human erythrocyte and Chinese hamster brain enzymes in the presence of PRPP gives a native molecular weight of 105,000 to 110,000 (subunit mol wt 26,000), again suggesting a tetramer. In the absence of PRPP, trimeric species of molecular weight ~78,000 are also observed.[85] PRPP appears to stabilize the molecule by enhancing its thermal stability.[59-61,71,76-78,85] Although vertebrate HGPRTases are likely tetrameric, the enzyme from yeast has been reported to be a monomer of mol wt 51,000.[64]

Despite the occurrence of a single type of polypeptide subunit, the native molecule exhibits at least three isoelectric species.[60,61,68,70,78] Three major forms (of identical catalytic characteristics) with PIs of 5.6, 5.7, and 5.9, and 6.2, 6.4, and 6.6 have been found for the human erythrocyte[60,78] and Chinese hamster[61] enzymes, respectively. This heterogeneity has led to the suggestion of isozymic forms of HGPRTase.[60,61,68,78] Since HGPRTase is encoded by a single gene on the X chromosome,[87] the isozymes has been proposed to be posttranslational alterations of a single gene product.[60,85,88] However, recent work with the human and Chinese hamster enzymes has shown that electrophoretic heterogeneity is eliminated by including the protease inhibitors phenylmethyl-sulfonyl-fluoride and 6-aminohexanoic acid as well as PRPP during purification.[85] Furthermore, HGPRTase from baker's yeast displays only one isoelectric species.[64]

Despite the overall physical and catalytic similarities of HGPRTase from different mammalian species, immunologic cross-reactions of antibodies to a given species do not occur with the enzyme from another species.[68,87] Since the amino acid composition of only human HGPRTase is known (Table 2), an explanation for the lack of cross-reactivity based on amino acid content cannot as yet be given. However, the immunological and uniqueness of mammalian HGPRTase does not prevent interspecies hybridization.[86] This suggests a preservation of the amino acid residues involved in subunit contacts.

C. Adenine Phosphoribosyltransferase (APRTase)

The second enzyme involved in purine nucleotide salvage is APRTase (EC 2.4.2.7; Figure 1, #2). The enzyme has been isolated to homogeneity from several mammalian tissues[89-93] as well as bacteria.[94] Characteristic of the PRTase family, the enzyme requires a divalent cation for activity. Cation specificity for mammalian APRTase appears broader, with Mg^{++}, Mn^{++}, and Ca^{++} being most effective, but still showing substantial activity with Co^{++}, Ni^{++}, Zn^{++}, and Ba^{++}.[92,95,96] The enzyme from *E. coli,* however, exhibits an absolute requirement for Mg^{++} or Mn^{++} and Ba^{++}, Ca^{++} and Zn^{++} are highly inhibitory.[94] APRTase from Ehrlich ascites tumor cells is the only PRTase where the binding of phosphoribosyl pyrophosphate has been extensively studied.[97] The binding of PRPP analogs has indicated that the 5-phosphate and 1-pyrophosphate groups are critical to binding and that the ribofuranose ring serves only as a spacer to keep these groups separated by a specific distance. Again, the Mg^{++} complex of PRPP is the biologically active form.[89,97-99]

The purine base specificity appears to be limited to adenine, 2,6-diamino purine and 4-amino 5-imidazole carboxamide (an intermediate in *de novo* purine biosynthesis.)[89,92,94,99] Studies with monkey liver APRTase have shown that substituents on the two position of the purine ring as well as ring nitrogen methylation significantly reduces binding. However, substitutions on the 6-amino group as well as ring position number 8 do not prevent binding.[99] Similar to HGPRTase, PRPP (and also adenine, AMP, and Mg^{++}-PPi) stabilizes the enzyme against thermal inactivation.[96-98] Chemical modification of APRTase has implicated both sulfhydryl and amino functions in the binding of PRPP, suggesting an ion pairing of a lysine residue with the phosphate groups of PRPP.[90,97]

Kinetic studies on APRTase from human erythrocytes,[89,96] *E. coli,*[94] and Ehrlich ascites cells[97,98] have suggested a Ping-Pong® type mechanism. Initially, PRPP is bound followed by the release of PPi, forming an enzyme-ribosylphosphate intermediate. Subsequent addition of adenine then releases AMP. Michaelis constants again vary with different organisms but range from 10^{-6} to 10^{-4} to 10^{-6} to 10^{-5} *M* for adenine and PRPP, respectively (Table 1c). The enzyme from mammals exhibits a broad pH optimum ranging from 6.5 to 10 (Table 1) with maximal turnover numbers ranging from 22 to 832 for pure enzyme.[89-91,94]

The molecular weight of the native APRTase molecule has been estimated from 20,000 to 45,000 from several mammalian species[89-93] as well as bacteria.[94,100] A single acidic polypeptide species (PI = 4.55, 4.85, and 5.65) of mol wt 11,000 to 18,000 has been isolated from human red blood cells[89,91] and rat liver,[90,93] respectively. The ambiguity in subunit composition of human erythrocyte APRTase has recently been resolved with a highly purified enzyme preparation. A native molecular weight of 38,200 and subunit molecular weight of 17,000 to 18,000 suggested a dimeric parent molecule. This was confirmed by cross-linking the enzyme with dimethylsuberimidate and peptide mapping.[91] However, the rat liver enzyme appears to be a monomer in the native state.[90] The amino acid composition of this purified enzyme (Table 2) shows a high content (33%) of hydrophobic amino acids. The cysteine content of six residues per dimer agrees favorably with the four residues per native molecule found for the rat liver enzyme.[90]

IV. PYRIMIDINE NUCLEOTIDE PHOSPHORIBOSYLTRANSFERASES

In contrast to the complex multienzyme system of purine nucleotide biosynthesis, the pyrimidine nucleotides are synthesized in a few relatively straightforward steps. In a sequence of three enzymatic reactions, the precursor molecules aspartate and carbamyl phosphate are condensed, cyclized, and oxidized to form orotic acid. The subsequent *de novo* step, catalyzed by orotate phosphoribosyltransferase (OPRTase), condenses orotic

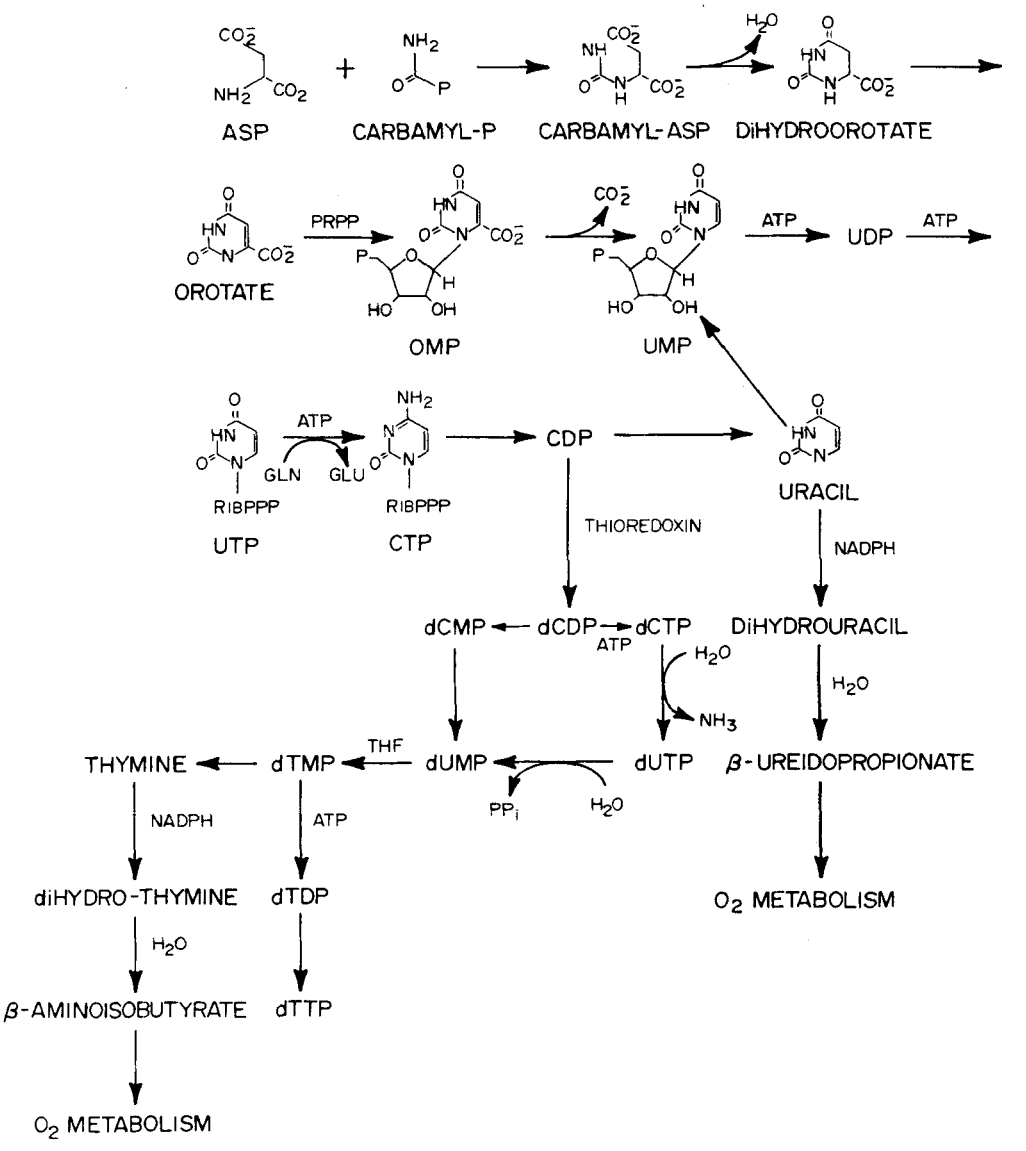


FIGURE 6. Pyrimidine nucleotide synthesis and interconversions.

acid with α-PRPP to form the primary pyrimidine nucleotide, orotidine 5′-phosphate (OMP). Further modification of OMP leads sequentially to the uridine, cytosine, and thymidine nucleotides.

Similar to the salvaging pathways of purine nucleotide metabolism, free pyrimidine bases are also recycled. In mammals, pyrimidine reutilization is catalyzed entirely by OPRTase and consequently this enzyme functions in both biosynthetic and salvaging operations. In addition to OPRTase, plants, bacteria, and yeast also contain a uracil-specific enzyme. The reactions of pyrimidine synthesis and salvage are shown in Figure 6.

A. Orotate Phosphoribosyltransferase (OPRTase)

OPRTase (EC 2.4.2.10) catalyzes the Mg^{++}-dependent reversible formation of orotidine 5′-phosphate from α-PRPP and orotic acid (Figure 1, #4). In all organisms thus far examined, OPRTase exhibits an absolute requirement for Mg^{++}[101-105] with optimal

activity achieved at ~5 m*M* Mg^{++}ion.[102,105] Studies with substrate analogs have demonstrated that the enzyme from human and beef erythrocytes requires a 2,4-diketo-substituted pyrimidine nucleus.[105] Other substrates utilized by OPRTase include 5-fluoroorotate, uracil, thymine, their 6-aza and 5-halo derivatives, xanthine, and, to a lesser extent, uric acid.[101,105,106] However, the specificity for orotate appears to be much greater in other organisms.[102,107,108]

Detailed kinetic studies of OPRTase in yeast in forward and reverse directions indicate that the reaction proceeds via a Ping-Pong®-type mechanism.[108] Similar to APRTase, the binding of PRPP results in an enzyme-phosphoribosyl intermediate followed by the release of PPi. The subsequent addition of orotate to this complex effectively constitutes a double displacement reaction which should proceed with retention of anomeric configuration. To rationalize the observed inversion of configuration, these authors have proposed that the enzyme stabilizes a ribose-phosphate carbonium ion. Other sugar phosphates tested were poorly bound to the enzyme. OPRTase from protozoans,[109] plants,[102] yeast,[103] and mammal[101,105,110] all exhibit a pH optimum of 7.5 to 9.0. Michaelis constants range from 10^{-6} to 10^{-4} (Table 1) for both orotate and PRPP and turnover numbers from 1320 to 2600 have been reported for the purified yeast enzyme (Table 1d).

OPRTase from all organisms studied thus far (except yeast) is found in vivo as a bifunctional enzyme complex with the next enzyme of the sequence, orotidylate decarboxylase (ODCase).[101,102,104-106,109-115] As a consequence of this association, OMP is channeled from OPRTase to ODCase without an appreciable accumulation of OMP.[112] Furthermore, as OMP is generated a conformational change is induced in the complex[106] which results in the stimulation of OPRTase activity.[112] This may provide a mechanism for conformational communication between sequential enzymes.

In the presence of guanidine·HCl, the OPRTase:ODCase complex from human erythrocytes may be separated into its individual components. The complex was found to be composed of an OPRTase dimer of mol wt 26,000 (subunit mol wt 13,000) and an ODCase dimer of mol wt 40,000 (subunit mol wt 20,000)[105,113] OPRTase activity in the absence of ODCase was dependent on the presence of exogenous thiols. Reconstitution of the complex resulted in the full restoration of both OPRTase and ODCase activities.

In yeast, OPRTase is not associated with ODCase.[103,108,116] However, like the mammalian enzyme, it is composed of two identical subunits of mol wt. ~20,000 each.[108] The activity of the yeast enzyme is not influenced by sulfhydryl blocking reagents.[103] The physical properties of OPRTase are given in Table 1.

B. Uracil Phosphoribosyltransferase (UPRTase)

In mammals, a single enzyme, OPRTase, is responsible for the salvage of pyrimidine bases. However, in bacteria,[117-119] yeast,[120,121] and plant cells[122] a uracil-specific enzyme is also found. UPRTase (EC 2.4.2.9; Figure 1, #5) catalyzes the synthesis of uridine monophosphate from uracil, α-PRPP and Mg^{++}[119,120,122] In pea seedlings, the divalent metal ion requirement is satisfied only by Mg^{++} and Mn^{++} is strongly inhibitory.[122] Again, the Mg^{++} complex of PRPP appears to be the active form.[121,122] In addition to uracil, the pea enzyme will also utilize 6-aza and 5-fluoro uracils, although at a much slower rate.

UPRTase from *E. coli* is a regulatory enzyme whose maximal activity is dependent on the presence of GTP or GDP.[117,119] The plant and yeast enzymes apparently are not affected by guanosine nucleotides.[120,122]

Kinetic studies on highly purified UPRTase from baker's yeast have suggested a Ping-Pong®-type reaction mechanism. Although the substrate binding order was not determined for the yeast enzyme, there is some evidence that *E. coli* UPRTase binds PRPP first.[117] Michaelis constants for uracil and PRPP have been determined for several organisms and vary from 10^{-6} to 10^{-5} *M* (Table 1c). The pH optimum for the yeast,[120]

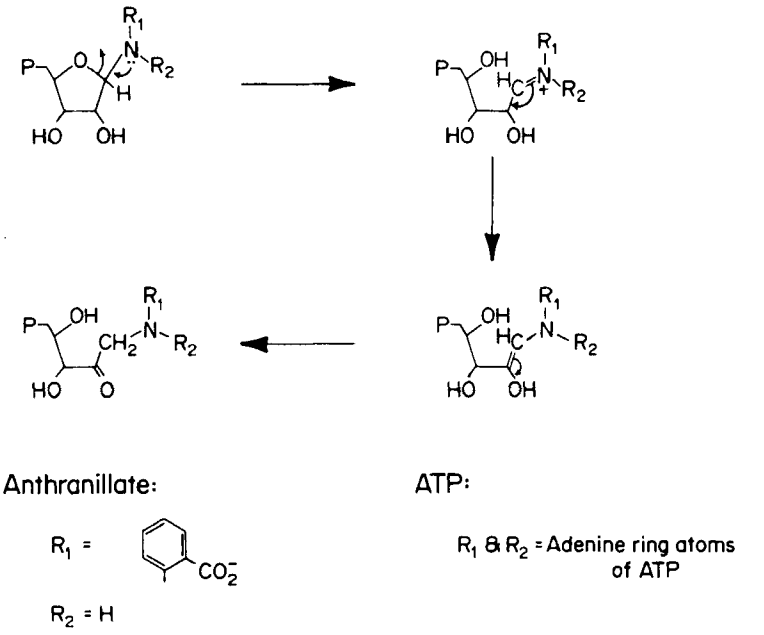


FIGURE 7. Amadori rearrangement of Trp and His biosynthesis.

plant,[122] and bacterial[117-119] enzymes ranges from 7.8 to 8.5. The turnover number for the pure yeast enzyme has been reported to be approximately 40. Similar to other PRTases, UPRTase is inhibited by mercurials. Since this inhibition is blocked by UMP, it has been suggested that the enzyme has an essential thiol(s) near the active center.[121] UMP has also been reported to enhance the thermal stability of the enzyme.[121]

The quaternary structure of UPRTase has been most extensively studied in bakers' yeast.[121] The molecular weight of the native enzyme was determined to be approximately 80,000 with two distinct subunit species of molecular weights 27,000 and 58,000. Furthermore, two isoenzymic species of the enzyme could be identified by gel electrophoresis and isoelectric focusing (PI = 5.27 and 5.35). Similar to the yeast enzyme, pea UPRTase has an apparent molecular weight of approximately 100,000[122] although the *E. coli* enzyme appears to be oligoromeric.[119]

V. PHOSPHORIBOSYLTRANSFERASES OF HISTIDINE AND TRYPTOPHAN BIOSYNTHESIS

Unlike most mammals which depend on a dietary supply of histidine and tryptophan, bacteria and other lower organisms are able to synthesize these amino acids *de novo*. The first enzymatic steps of His and Trp synthesis are catalyzed by separate Mg^{++}-dependent PRTases which utilize ATP and anthranilate, respectively, as nitrogenous ligands. The products of these reactions, phosphoribosyl-ATP and phosphoribosyl-anthranilate, then undergo a complex rearrangement (Amadori rearrangement, see Figure 7) which opens the phosphoribosyl ring. In the next series of reactions, various carbon atoms of the ribose skeleton are degraded or modified such that two of these atoms are incorporated into the tryptophan molecule and all five retained in histidine. Hence, in contrast to nucleotide synthesis and salvage, the ribosylphosphate moiety of PRPP is utilized as a source of structural carbon.

A. ATP-Phosphoribosyltransferase (ATP-PRTase)

ATP-PRTase (EC 2.4.2.17; Figure 1, #9) is the first enzyme in the biosynthetic pathway of histidine. It catalyzes a divalent metal-dependent condensation of α-PRPP with the number 1 adenine ring nitrogen of ATP to form phosphoribosyl-ATP (PRib-ATP). Similar to other PRTases, Mg^{++} or Mn^{++} are the most effective cations and the Mg^{++} complexes of both substrates are believed to be the catalytically active forms.[123-125] Other cations such as Zn^{++}, Ag^{++}, Cd^{++}, Ni^{++}, Cu^{++}, and Co^{++}, as well as high concentrations of Mg^{++} strongly inhibit the enzyme.[123,126] Substrate analog studies with highly purified ATP-PRTase from *S. typhimurium* indicate that the enzyme is specific for ATP and PRPP although ADP and AMP compete for the ATP binding site.[126] Initial velocity and product inhibition studies in the forward (biosynthetic) and reverse directions have suggested an ordered bibi mechanism where ATP is bound before PRPP and PRib-ATP is released after PPi.[123,124,127] The equilibrium constant for the reaction does not favor the synthesis of PRib-ATP and it has been suggested that the forward reaction is facilitated by the hydrolysis of PPi by pyrophosphatases.[126] Michaelis constants for both substrates range from 10^{-5} to 10^{-4} (Table 1c) at an optimal pH of ~8.5.[128] Turnover numbers for the *S. typhimurium* enzyme ranging from 862 to 1148 (Table 1c)[127,129] have been reported.

Similar to other PRTases, ATP-PRTase is stabilized by thiol reagents.[126,129,130] The enzyme contains a total of five cysteine residues per subunit (Table 2), none of which are buried.[130] Chemical modification of these residues with mercurials inactivates the protein and results in a disaggregation of the subunits.[126,130] Other stabilizers of ATP-PRTase include histidine, Na^{+}, Mg^{++}, and ATP.[126,129,130]

In most organisms, ATP-PRTase is a complex regulatory protein composed of six identical subunits of mol wt 33,000 to 35,000.[129,131,132] It is allosterically inhibited by the biosynthetic end product histidine,[126,127,129,130,133] AMP,[134] and also by guanosine tetraphosphate synergistically with histidine.[135] Studies with the normally dimeric ATP-PRTase from *E. coli* have demonstrated that His and AMP cause the enzyme to associate into a hexamer. Each dimeric species (mol wt ~67,000) was shown to bind a single His molecule.[134] In other systems, each subunit binds one His molecule (in the hexameric form) generating a conformational change in the enzyme.[126,127,133,136]

In addition to the substrate and allosteric modulator binding sites, ATP-PRTase binds histidyl-tRNA.[127,137] Complexation studies with the *S. typhimurium* enzyme have demonstrated, however, that only 0.3 molecules of tRNA are bound per hexamer.[137] ATP-PRTase is an autoregulatory protein which regulates the expression of its own gene through direct interaction with histidine operator attenuator DNA.[138-141]

The complete amino acid sequence of ATP-PRTase from *S. typhimurium* has recently been determined by automated Edman degradation and DNA sequence data.[132] The enzyme subunit is comprised of 299 amino acids with no apparent regions of high hydrophobic or hydrophilic character. However, near the amino terminus of the enzyme, a cluster of basic residues followed by a cluster of acidic residues was found. In the section on QPRTase, it was noted that a linear sequence of nine residues containing an active center lysine of QPRTase was also found in ATP-PRTase. This suggests that residues 143 to 150 of ATP-PRTase are near the active center of the enzyme. Interestingly, a sequence of 40 residues of ATP-PRTase was observed to contain 13 residues which could be aligned with corresponding residues in the sequence of glyceraldehyde-3-phosphate dehydrogenase near the active center cysteine.

B. Anthranilate Phosphoribosyltransferase (Anth-PRTase)

Anth-PRTase (EC 2.4.2.18; Figure 1, #10) catalyzes the second reaction in the biosynthetic pathway of Trp. Like ATP-PRTase it is found only in bacteria and lower organisms.[144] Three distinct molecular species of Anth-PRTase have been identified in

different bacterial genera. The smallest of these, found in the enteric bacterium *Serratia marcescens,* is monomeric and has a molecular weight of ~45,000.[145] A second form, typified by the species *Erwinia carotovora,* is a dimeric molecule with a subunit molecular weight of ~40,000.[146] The largest form of the enzyme also exhibits glutamine amidotransferase activity (the first enzyme of the pathway) and is a dimer with a subunit molecular weight of 65,000.[147-149] Glutamine amidotransferase of *Serratia* has a molecular weight of approximately 20,000 and a gene fusion with Anth-PRTase has been postulated to have generated the bifunctional species of *Escherichia coli* and *Salmonella.*[150-152]

Anth-PRTase catalyzes the formation of a β-amino glycoside with the free amino group of anthranilate and PRPP. The reaction is specific for PRPP[153] and typically Mg^{++}-dependent[147,148,153,154] although the enzyme is also active with Mn^{++} and Co^{++}.[154] Kinetic studies on the monomeric and dimeric aggregate of *Salmonella typhimurium* Anth-PRTase suggest an ordered sequential mechanism where PRPP is the first substrate bound.[147] However, the pattern of PPi product inhibition varied with the aggregation state of the enzyme. Michaelis constants for anthranilate and PRPP were essentially identical for the monomeric and dimeric forms with magnitudes of approximately 10^{-6} *M* (Table 1c). Turnover numbers for the different species of pure enzyme range from 85 to 1641[145-148] at a pH optimum of ~7.5 to 8.0 (Table 1d).[145,147,148]

Chemical modification of cysteine residues strongly inhibits Anth-PRTase from *Aerobacter aerogenes*[153] and *S. typhimurium.*[155] Furthermore, Anth-PRTase from *S. typhimurium* exhibits a pyridoxal-phosphate reactive lysine associated with the PRPP binding site.[156] The enzyme is also allosterically inhibited by tryptophan, the metabolic end product of the pathway.[147,148,153,154] Similar to the interaction of histidine with ATP-PRTase, Trp produces a conformational change in Anth-PRTase.[154]

N-terminus sequencing of *Erwinia* and *Serratia* Anth-PRTase has revealed a considerable homology.[146] The first residue of the *Serratia* sequence corresponded to the fourteenth residue of *Erwinia* and 73% of the remaining 26 sequenced residues were idential. The extra *N*-terminal *Erwinia* residues have been implicated to be involved in dimer formation.[146] Homologies with sequence data from the other PRTases were not apparent.

VI. FEATURES OF THE PHOSPHORIBOSYLTRANSFERASE FAMILY

Similarities in the physical and catalytic properties of the PRTases suggest that they may share common structural features. These features could be dictated by chemical constraints imposed by the substrate molecules (convergent evolution), or, more likely, they may represent structural information inherited from a common ancestral protein (divergent evolution). An inspection of Table 1 shows that PRTase subunit molecular weights range from 13,000 (OPRTase) to approximately 53,000 (Gln-aPRTase), corresponding to ~118 to 482 amino acids. Therefore, a minimal "core" protein exhibiting PRTase activity should contain approximately 118 residues and additions to this basic structure should be associated with the binding of various other substrates, prosthetic groups, or cofactors. From the previous sections it can be seen that only QPRTase, HGPRTase, APRTase, and OPRTase are purely phosphoribosyltransferases, which do not bind regulatory molecules or cofactors. Furthermore, this group exhibits the lowest molecular weights of the PRTases ranging from 13,000 to 28,000. The remaining enzymes of the PRTase family interact with one or more additional molecules and have higher molecular weights (40,000 to 53,000). It is also noteworthy that QPRTase, which catalyzes a reaction similar to that catalyzed by the OPRTase:ODCase complex, has a molecular weight very nearly the sum of the complex components (13,000

and 20,000 respectively). QPRTase may, therefore, represent a covalently linked bifunctional enzyme arising from a gene fusion as observed with the amidotransferase property of Anth-PRTase.

Where the information is available, the PRTases are found to be acidic proteins with generally alkaline pH optima (Table 1b). Since cysteine appears to be involved in PRTase catalysis (Table 1d), the alkaline pH optimum may be a reflection of the participation of a thiolate anion in the mechanism. Recent studies on kinetic isotope effects in ATP-PRTase, OPRTase, and HGPRTase have indicated that β glycoside formation proceeds through an SN1 carbocation reaction involving a carbonium ion.[158] Furthermore, it has been proposed that both APRTase and OPRTase stabilize carbonium ion intermediates.[98,108] This stabilizing function, which may be performed by an ionized cysteine residue, is consistent with the requirement for exogenous sulfhydryl reagents observed for most of the PRTases and the substrate protection against mercurial inhibition for several.

In addition to the cysteine residue(s) required for PRTase catalysis, lysine also appears to be associated with the binding of substrate. At the moderately alkaline pH optima characteristic of these enzymes, lysine residues are protonated and the role of the essential lysines may, therefore, be to ion-pair with the phosphates of the common substrate PRPP. This is consistent with the identification of a common active center lysyl peptide in both QPRTase and ATP-PRTase. This further suggests that the amino acid sequence near the substrate binding sites of the other PRTases may also be conserved.

Kinetically, the PRTases appear to favor an ordered sequential mechanism of glycoside formation where α-PRPP is bound to the enzyme first. However, at least two of these enzymes utilize a Ping-Pong®-type mechanism involving an enzyme-ribosylphosphate intermediate. Since the implied double displacement of a Ping-Pong® reaction would lead to a retention of configuration, it has been proposed that a carbonium ion intermediate is common to both types of mechanism.[158] From Table 1c it can be seen that the Michaelis constants for both substrates do not reflect mechanistic differences. Furthermore, the K_ms observed for a given PRTase generally vary as much between species as between different members of the enzyme family ($\sim 10^{-6}$ to 10^{-4} M).

A striking feature of PRTase catalysis is their collective low turnover number (Table 1d). Expressed on an s^{-1} basis, values ranging from $<<1$ up to 43 are obtained. This compares with an average of about 10^2 s^{-1} for intracellular enzymes and is far below enzymes such as catalase and carbonic anhydrase which operate at $>10^5$ s^{-1}.[159] Again, this characteristic could be a constraint imposed by the chemistry involved or a common inherited feature.

A reason for the acidic nature of the PRTases is not evident from the amino acid compositions listed in Table 2. The percentage of both acidic and basic residues of these five PRTases compares well with the "average" content of most proteins. However, the content of hydrophobic residues is consistently higher than average and is largely due to the greater leucine contribution. Since leucine favors α-helix formation[160] one might speculate that the PRTases also have a greater than average α-helix content. Alternatively, the hydrophobicity of the PRTases may be a reflection of the nature of enzyme-substrate interaction.

Since all of the PRTases are specific for PRPP, each of the enzymes may utilize a common architectural principle in the binding of this substrate. The conserved lysyl peptide of QPRTase and ATP-PRTase may be a reflection of such a principle. Furthermore, since a mononucleotide is generally the product of each enzyme, the structural features of a PRPP binding fold might also be expected to accommodate the binding of a nucleoside monophosphate. Consequently, a PRPP fold might exhibit a topological homology to the mononucleotide domain observed in various dehydro-

genases and kinases.[161,162] This super-secondary structural grouping of three parallel strands of β-pleated sheet and two α-helices has been recognized as binding a variety of mononucleotides as well as aromatic dye analogs.[163,164] Recent work with yeast OPRTase has demonstrated that the enzyme is tightly bound to cibacron blue-linked Sepharose® and is specifically eluted by PRPP and OMP.[116] Since this behavior has been regarded as diagnostic for the presence of a mono- or dinucleotide fold,[125] OPRTase may contain a similar structural feature. In a similar fashion, QPRTase is bound and specifically eluted by substrate from fluorescein dye-linked Sepharose®.[165] Furthermore, salicylate has been found to be a competitive inhibitor of nicotinate ($K_i = 1.6 \times 10^{-4} M$) in NPRTase.[8] Since both salicylate and fluorescein dyes have been demonstrated to bind in a hydrophobic pocket in the nucleotide fold,[166-168] related structural features may also occur in QPRTase and NPRTase.

VII. MOLECULAR DISEASES OF THE PHOSPHORIBOSYLTRANSFERASES

Several inherited metabolic disorders in man are known to be caused by abnormalities of the purine and pyrimidine phosphoribosyltransferases. The clinical symptomology of these disorders is highly variable and reflects, in part, the marked heterogeneity of molecular lesions affecting these enzymes. Defects in physical and catalytic properties as well as in rates of synthesis have been documented for the PRTases of these metabolic pathways. Since UPRTase is not found in man, the mutations of OPRTase affect salvage and *de novo* synthesis. (See Section IV. A., B.). However, of the purine nucleotide PRTases, only the salvaging enzymes HGPRTase and APRTase appear to have demonstrated mutations. Abnormalities of the pyridine nucleotide PRTases have not been observed.

A. The HGPRTase Deficiencies

The complete or near complete lack of measurable HGPRTase activity in all tissues is associated with a debilitating neurologic disease known as the Lesch-Nyhan syndrome.[169] The disease in its most severe forms is clinically characterized by irregular, spastic and athetoid movements, retardation, and a unique compulsion for self-mutilation. Individuals with this disease excrete excessive amounts of uric acid and tissue deposition of urate salts may lead to renal failure and arthritic gout. The overproduction of uric acid is the result of an increased rate of *de novo* purine biosynthesis. This is believed to be caused by the greater availability of PRPP (due to its decreased utilization by HGPRTase) for the first step of *de novo* synthesis catalyzed by Gln-aPRTase. Furthermore, in the absence of the physiological tissue levels of GMP and IMP, feedback control of Gln-aPRTase may be inhibited.[87]

Early immunologic studies suggested that some patients with classic Lesch-Nyhan disease produce normal quantities of catalytically inactive and electrophoretically altered HGPRTase.[170-172] Although this is consistent with a mutation in a structural gene, the absence of CRM^+ material in other patients argues that the rate of HGPRTase synthesis has been affected. However, this lack of cross-reactivity is currently believed to be due to structural alterations in the antigenic sites of the molecule.[217,218]

A second category of mutants exhibiting a partial deficiency of HGPRTase (~1% to 30% normal) is also characterized by gouty arthritis and renal damage, but self-inflicted mutilation does not occur.[173] Since brain tissue is particularly dependent on the purine salvage pathways,[174,175] the higher levels of HGPRTase found in these patients may retard the accumulation of toxic metabolites[174,176-178] or the depletion of intermediates or cofactors necessary for normal central nervous system (CNS) function.[87,179]

The examination of HGPRTase levels in gouty subjects has revealed a multitude of molecular defects associated with partial deficiency. Changes in the physical properties of the enzyme such as increased[181] or decreased[182-184] thermal sensitivity have been correlated with changes in electrophoretic mobility.[184] A mutation reducing the in vivo stability of the enzyme has also been reported.[185] A large number of kinetic variants have been observed affecting sensitivity to product inhibition,[184,186] substrate specificity,[181] and substrate affinities for PRPP as well as hypoxanthine.[183,187,188] Alterations in electrophoretic properties are also associated with these changes in Michaelis constants.[187,188] Evidence for changes in the antigenic determinants of the HGPRTase molecule comes from the lack of CRM^+ material in some patients with partial deficiency.[189,190]

HGPRTase characterized from Lesch-Nyhan patients as well as those with the partial deficiencies suggests that the HGPRTase structural gene is highly mutable. The HGPRTase gene has been located in the X chromosome and the abnormal traits are recessive in character.[87] Since Lesch-Nyhan patients do not survive long enough to reproduce, this severe form of HGPRTase deficiency affects only males.

B. The APRTase Deficiencies

Similar to HGPRTase, both partial and complete deficiencies of APRTase have been demonstrated in man.[173,191,192] Erythrocyte APRTase activities in partially deficient individuals range from 8 to 45% of normal,[173,191] although clinically most patients are asymptomatic. Despite the generally benign nature of the partial deficiency, hyperuricemia, gout, and tissue deposits of urate may also be associated with the disorder.[191,193-195] Population screening of normouricemic and hyperuricemic individuals has identified at least two classes of partial deficiencies.[191] One group was characterized by a decreased half-life of peripheral erythrocyte APRTase while a second group exhibited a selective reduction in leucocyte activity. Alterations in charge, Stokes radius, and kinetic properties were not observed. The frequency of partial APRTase deficiency has been estimated to be about 1%.[191]

Thus far, there have only been six reported cases of complete APRTase deficiency.[192,198-200] The defect is clinically manifested by elevated levels of urinary adenine and 8-hydroxyadenine as well as urolithiasis due to 2,8-dihydroxyadenine stone formation. Presumably tissue levels of adenine, which are increased due to the lack of APRTase, and further oxidized to the insoluble hydroxyadenines by xanthine oxidase.[192] The nature of the molecular defect(s) in complete APRTase deficiency has not been determined. Pedigrees of families with the complete deficiency indicate an autosomal recessive mode of disease transmission.[192,198-200]

C. Abnormalities of the OPRTase:ODCase Complex

In contrast to the purine nucleotide pathways, a single phosphoribosyltransferase, OPRTase, is involved in both the *de novo* synthesis and salvage of the pyrimidine nucleotides in man. As previously described, human OPRTase is found as a tightly associated bifunctional complex with the subsequent enzyme of the pathway, ODCase. Two types of defects have been identified in this complex which result in the near complete deficiency of both OPRTase and ODCase activities (type I) or of ODCase alone (type II). Clinically, both of these disorders are indistinguishable and are characterized by poor growth, megaloblastosis associated with hypochromic anemia, and excessive amounts of orotic acid excreted in the urine.[202] Residual OPRTase activity in the type I disorder and the normal OPRTase activity of the type II disorder also result in elevated urinary orotidine. Similar to the HGPRTase deficiencies, both of these disorders are associated with an accelerated rate of *de novo* pyrimidine biosynthesis.[202] This has been

proposed to result from a reduction in feedback inhibition of carbamyl phosphate synthetase by the pyrimidine nucleotides or by activation of the enzyme by PRPP.[203] Since orotic acid itself is not a toxic metabolite, etiologically the metabolic disorder is believed to result from a pyrimidine starvation with subsequent perturbations to normal nucleic acid and cofactor synthesis.[202]

Studies on the properties of the complex from a type I patient revealed a change in electrophoretic mobility and thermal stability.[204] This suggests a structural gene mutation in one or both of the complex enzymes. Due to the tight association of these enzymes in man and their instability when separated, a structural change in one could influence the activity of the other. The specific defects affecting OPRTase or ODCase separate from the complex have not yet been identified. Like the APRTase deficiencies, abnormalities of the OPRTase:ODCase complex are autosomally transmitted.

D. Phosphoribosylpyrophosphate Synthetase (PRPP Synthetase)

In view of the discussion in "Features of the Phosphoribosyltransferase Family" that a PRPP binding fold might be a common structural feature of the PRTases, it is interesting to examine some of the properties of the enzyme which synthesizes PRPP. PRPP synthetase catalyzes the divalent metal and phosphate-dependent formation of PRPP from ATP and ribose-5-P.[205-207] The enzyme from human erythrocytes[205] and rat liver[206] is highly specific for both substrates with Michaelis constants on the order of 10^{-5} to 10^{-4} *M* at a pH optimum of approximately 7.2 to 8.8. The native enzyme is an oligomer of identical subunits of molecular weight 32,000 to 40,500,[205,206,208] and an isoelectric point of 4.7 to 5.1.[205,206,208] Similar to the PRTases, it is inactivated by sulfhydryl modifying reagents and stabilized by mercaptans.[209] Kinetically, the enzyme proceeds via an ordered bibi mechanism where ribose-5-P is bound first and PRPP is released last.[210] PRPP synthetase is a complex regulatory enzyme which is inhibited by a variety of purine, pyrimidine, and pyridine nucleoside phosphates and 2,3-diphosphoglycerate.[209,210]

Although deficiency disorders of PRPP synthetase have not been observed, a number of defects yielding a hyperactive enzyme have been reported. Since tissue levels of PRPP partially govern the rate of *de novo* purine biosynthesis in man,[211] overproduction of PRPP leads to an acceleration of purine synthesis with concomitant appearance of gouty symptomology. The hyperactivity of these PRPP synthetase variants is a consequence of mutations in both catalytic and regulatory sites of the molecule.[208,212,213] Structural alterations affecting an increased activity per enzyme molecule, change in electrophoretic mobility, and decrease in thermal stability have been identified in one family.[208] A second kinetic variant is characterized by an increased affinity for ribose-5-P and, hence, is catalytically more active than the normal enzyme at a given level of ribose-5-P.[213] As described above, PRPP synthetase activity is subject to feedback inhibition by nucleotides and 2,3-diphosphoglycerate. A regulatory mutant has been described where the sensitivity of the enzyme to these inhibitors is markedly reduced although the kinetic properties of the enzyme are unchanged.[212] The pedigrees of these PRPP synthetase variants suggest an X-linked mode of transmission.[212,214]

VIII. SIGNIFICANCE

The study of protein tertiary structures has revealed that functionally related proteins are frequently topologically similar.[162] This observation has led to the concept that function may dictate structure (convergent evolution) or that structural and functional homology reflects a common evolutionary origin (divergent evolution). Evidence has been presented suggesting that the PRTases constitute a class of functionally and perhaps

structurally related proteins. Since neither complete amino acid sequence nor tertiary structural information is yet available for the PRTases, potential evolutionary relationships can only be inferred from similarities in their physical-chemical properties and from limited sequence homologies. The significance of these similarities is underscored by the high incidence of structural abnormalities in the PRTases and PRPP synthetase. This suggests that those structural features responsible for binding PRPP share either a common instability to random mutation or enhanced acceptance of mutational events. The former possibility would, therefore, imply the existence of a "tight" or efficient catalytic domain which tolerates few perturbations to its structure. Alternatively, a greater acceptance of random mutation could suggest that the PRTases play less crucial metabolic roles and hence are less stringently selected against. Since convergent evolution to either possibility is unlikely, it is proposed that the PRTases arose divergently from an ancestral protein associated with the binding of PRPP. Furthermore, it is suggested that the PRTase deficiency disorders are a consequence of mutations to a common structural domain.

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REFERENCES

1. **Ogasawara, N. and Gholson, R. K.,** The non-identity of yeast nicotinic acid mononucleotide pyrophosphorylase and nicotinic acid mononucleotidase, *Biochim. Biophys. Acta,* 118, 422, 1966.
2. **Honjo, T., Nakamura, S., Nishizuka, Y., and Hayaishi, O.,** Stoichiometric utilization of adenosine 5'-triphosphate in nicotinate ribonucleotide synthesis from nitotinate and 5-phosphoribosyl-l-pyrophosphate, *Biochem. Biophys. Res. Commun.,* 25, 199, 1966.
3. **Kosaka, A., Spivey, H. O., and Gholson, R. K.,** Nicotinate phosphoribosyltransferase of yeast. Purification and properties, *J. Biol. Chem.,* 246, 3277, 1971.
4. **Kosaka, A., Spivey, H. O., and Gholson, R. K.,** Yeast nicotinic acid phosphoribosyltransferase. Studies of reaction paths, phosphoenzyme, and Mg^{2+} effects, *Arch. Biochem. Biophys.,* 179, 334, 1977.
5. **Seifert, R., Kittler, M., and Hilz, H.,** Purification and characterization of nicotinate phosphoribosyltransferase from Ehrlich ascites carcinoma cells, in *Current Aspects of Biochemical Energetics,* Kaplan, N. O. and Kennedy, E. P., Eds., Academic Press, New York, 1966, 413.
6. **Kahn, V. and Blum, J. J.,** ATP-independent nicotinic acid mononucleotide pyrophosphorylase of *Astasia longa, Biochim. Biophys. Acta,* 146, 305, 1967.
7. **Smith, L. D. and Gholson, R. K.,** Allosteric properties of bovine liver nicotinate phosphoribosyltransferase, *J. Biol. Chem.,* 244, 68, 1969.
8. **Gaut, Z. N. and Solomon, H. M.,** Inhibition of nicotinate phosphoribosyltransferase by nonsteroidal anti-inflammatory drugs: a possible mechanism of action, *J. Pharm. Sci.,* 60, 1887, 1971.
9. **Niedel, J. and Dietrich, L. S.,** Nicotinate phosphoribosyltransferase of human erythrocytes. Purification and properties, *J. Biol. Chem.,* 248, 3500, 1973.
10. **Imsande, J.,** A comparative study of the regulation of pyridine nucleotide formation, *Biochim. Biophys. Acta,* 82, 445, 1964.
11. **Baecker, P. A., Yung, S. G., Rodriguez, M., Austin, E., and Andreoli, A. J.,** Periplasmic localization of nicotinate phosphoribosyltransferase in *Escherichia coli, J. Bacteriol.,* 133, 1108, 1978.
12. **Dudley, M. A. and Willett, H. P.,** Nicotinamide adenine dinucleotide synthesis by *Mycobacterium tuberculosis* var. bovis, *Proc. Soc. Exp. Biol. Med.,* 119, 807, 1965.
13. **Sundaram, T. K., Rajagopalan, K. V., Pichappa, C. V., and Sarma, P. S.,** Studies on pyridine nucleotide biosynthesis, *Biochem. J.,* 77, 145, 1960.
14. **Preiss, J. and Handler, P.,** Biosynthesis of diphosphopyridine nucleotide. 1. Identification of intermediates, *J. Biol. Chem.,* 233, 488, 1958.

15. **Dietrich, L. S., Fuller, L., Yero, I. L., and Martínez, L.,** Nicotinamide mononucleotide pyrophosphorylase activity in animal tissues, *J. Biol. Chem.*, 241, 188, 1966.
16. **Powanda, M. C., Muniz, O., and Dietrich, L. S.,** Studies on the mechanism of rat liver nicotinamide mononucleotide pyrophosphorylase, *Biochemistry*, 8, 1869, 1969.
17. **Lin, L. F. H., Lan, S. J., Richardson, A. H., and Henderson, L. M.,** Pyridine nucleotide synthesis. Purification of micotinamide mononucleotide pyrophosphorylase from human erythrocytes, *J. Biol. Chem.*, 247, 8016, 1972.
18. **Dietrich, L. S., Fuller, L., Yero, I. L., and Martinez, L.,** Nicotinamide mononucleotide pyrophosphorylase activity in ascites cell extracts, *Nature (London)*, 208, 347, 1965.
19. **Ijichi, H., Ichiyama, A., and Hayaishi, O.,** Studies on the biosynthesis of nicotinamide adenine dinucleotide. III. Comparative *in vivo* studies on micotinic acid, nicotinamide, and quinolinic acid as precursors of nicotinamide adenine dinucleotide, *J. Biol. Chem.*, 241, 3701, 1966.
20. **Nishizuka, Y. and Hayaishi, O.,** Studies on the biosynthesis of nicotinamide adenine dinucleotide. I. Enzymic synthesis of niacin ribonucleotides from 3-hydroxyanthranilic acid in mammalian tissues, *J. Biol. Chem.*, 238, 3369, 1963.
21. **Hadwiger, L. A., Badiei, S. E., Waller, G. R., and Gholson, R. K.,** Quinolinic acid as a precursor of nicotinic acid and its derivatives in plants, *Biochem. Biophys. Res. Commun.*, 13, 466, 1963.
22. **Andreoli, A. J., Ikeda, M., Nishizuka, Y., and Hayaishi, O.,** Quinolinic acid: a precursor to nicotinamide adenine dinucleotide in *Escherichia coli, Biochem. Biophys. Res. Commun.*, 12, 92, 1963.
23. **Konno, K., Oizumi, K., and Oka, S.,** Biosynthesis of niacin ribonucleotide from quinolinic acid by mycobacteria, *Nature (London)*, 205, 874, 1965.
24. **Packman, P. M. and Jakoby, W. B.,** Crystalline quinolinate phosphoribosyltransferase, *J. Biol. Chem.*, 240, PC4107, 1965.
25. **Packman, P. M. and Jakoby, W. B.,** Crystalline quinolinate phosphoribosyltransferase. II. Properties of the enzyme, *J. Biol. Chem.*, 242, 2075, 1967.
26. **Iwai, K., Shibata, K., Taguchi, H., and Itakura, T.,** Properties of crystalline quinolinate phosphoribosyltransferase from *Alcaligenes eutrophus* nov. subsp. *quinolinicus, Agric. Biol. Chem.*, 43, 345, 1979.
27. **Taguchi, H. and Iwai, K.,** The isolation and physico-chemical properties of crystalline quinolinate phosphoribosyltransferase from hog liver, *Agric. Biol. Chem.*, 39, 1493, 1975.
28. **Mann, D. F. and Byerrum, R. U.,** Quinolinic acid phosphoribosyltransferase from castor bean endosperm. I. Purification and characterization, *J. Biol. Chem.*, 249, 6817, 1974.
29. **Taguchi, H. and Iwai, K.,** Characteristics of quinolinate phosphoribosyltransferase from the "Shiitake" mushroom *(Lentinus edodes), J. Nutr. Sci. Vitaminol.*, 20, 283, 1974.
30. **Gholson, R. K., Ueda, I., Ogasawara, N., and Henderson, L. M.,** The enzymatic conversion of quinolinate to nicotinic acid mononucleotide in mammalian liver, *J. Biol. Chem.*, 239, 1208, 1964.
31. **Nakamura, S., Ikeda, M., Tsuji, H., Nishizuka, Y., and Hayaishi, O.,** Quinolinate transphosphoribosylase: a mechanism of niacin ribonucleotide formation from quinolinic acid, *Biochem. Biophys. Res. Commun.*, 13, 285, 1963.
32. **Taguchi, H. and Iwai, K.,** Inhibition of hog liver crystalline quinolinate phosphoribosyltransferase by nucleotides, quinolinate analogues and sulfhydryl reagents, *Agric. Biol. Chem.*, 40, 385, 1976.
33. **Suzuki, N., Carlson, J., Griffith, G., and Gholson, R. K.,** Studies on the *de novo* biosynthesis of NAD in *Escherichia coli*. I. Properties of the quinolinic acid synthetase system, *Biochim. Biophys. Acta*, 304, 309, 1973.
34. **Ogasawara, N., Chandler, J. L. R., Gholson, R. K., Rosser, R. J., and Andreoli, A. J.,** Biosynthesis of quinolinic acid in a cell-free system, *Biochim. Biophys. Acta*, 141, 199, 1967.
35. **Wicks, F. D., Sakakibara, S., Gholson, R. K., and Scott, T. A.,** The mode of condensation of aspartic acid and dihydroxyacetone phosphate in quinolinate synthesis in *Escherichia coli, Biochim. Biophys. Acta*, 500, 213, 1977.
36. **Taguchi, H. and Iwai, K.,** Properties of the crystalline quinolinate phosphoribosyltransferase from hog liver, *Agric. Biol. Chem.*, 39, 1599, 1975.
37. **Iwai, K., Shibata, K., and Taguchi, H.,** Crystalline quinolinate phosphoribosyltransferase from hog liver: the molecular weight and some enzymic properties, *Agric. Biol. Chem.*, 43, 351, 1979.
38. **Musick, W. D. L.,** unpublished results, Purdue University, W. Lafayette, Ind., 1979.
39. **Taguchi, H. and Iwai, K.,** Chemical modifications of the crystalline quinolinate phosphoribosyltransferase from hog liver, *Biochim. Biophys. Acta*, 422, 29, 1976.
40. **Mahoney, W. A.,** unpublished results, Purdue University, West Lafayette, Ind., 1979.
41. **Musick, W. D. L.,** Preliminary crystallographic studies on quinolinate phosphoribosyltransferase, *J. Mol. Biol.*, 117, 1101, 1977.
42. **Rottman, F. and Guarino, A. J.,** The inhibition of phosphoribosylpyrophosphate amidotransferase activity by cordycepin monophosphate, *Biochim. Biophys. Acta*, 89, 465, 1964.

43. **Nierlich, D. P. and Magasanik, B.,** Regulation of purine ribonucleotide synthesis by end product inhibition, *J. Biol. Chem.*, 240, 358, 1965.
44. **Momose, H., Nishikawa, H., and Katsuya, N.,** Genetic and biochemical studies on 5'-nucleotide fermentation. II. Repression of enzyme formation in purine nucleotide biosynthesis in *Bacillus subtilis* and derivation of derepressed mutants, *J. Gen. Appl. Microbiol.*, 11, 211, 1965.
45. **Wong, J. Y., Meyer, E., and Switzer, R. L.,** Glutamine phosphoribosylpyrophosphate amidotransferase from *Bacillus subtilis*, *J. Biol. Chem.*, 252, 7424, 1977.
46. **Messenger, L. J. and Zalkin, H.,** Glutamine phosphoribosylpyrophosphate amidotransferase from *Escherichia coli*. Purification and properties, *J. Biol. Chem.*, 254, 3382, 1979.
47. **Nagy, M.,** Regulation of the biosynthesis of purine nucleotides in *Schizosaccharomyces pombe*. 1. Properties of the phosphoribosylpyrophosphate: glutamine amidotransferase of the wild strain and of a mutant desensitized towards feedback modifiers, *Biochim. Biophys. Acta*, 198, 471, 1970.
48. **Rowe, P. B. and Wyngaarden, J. B.,** Glutamine phosphoribosylpyrophosphate amidotransferase. Purification, substructure, amino acid composition, and absorption spectra, *J. Biol. Chem.*, 243, 6373, 1968.
49. **Wood, A. W. and Seegmiller, J. E.,** Properties of 5-phosphoribosyl-l-pyrophosphate amidotransferase from human lymphoblasts, *J. Biol. Chem.*, 248, 138, 1973.
50. **Holmes, E. W., McDonald, J. A., McCord, J. M., Wyngaarden, J. B., and Kelley, W. N.,** Human glutamine phosphoribosylpyrophosphate amidotransferase. Kinetic and regulatory properties, *J. Biol. Chem.*, 248, 144, 1973.
51. **Holmes, E. W., Wyngaarden, J. B., and Kelley, W. N.,** Human glutamine phosphoribosylpyrophosphate amidotransferase. Two molecular forms interconvertible by purine ribonucleotides and phosphoribosylpyrophosphate, *J. Biol. Chem.*, 248, 6035, 1973.
52. **Singer, S. C. and Holmes, E. W.,** Human glutamine phosphoribosylpyrophosphate amidotransferase. Hysteretic properties, *J. Biol. Chem.*, 252, 7959, 1977.
53. **King, G. L., Bounous, C. G., and Holmes, E. W.,** Human placental amidophosphoribosyltransferase. Comparison of the kinetics of glutamine and ammonia utilization, *J. Biol. Chem.*, 253, 3933, 1978.
54. **Itakura, M. and Holmes, E. W.,** Human amidophosphoribosyltransferase. An oxygen-sensitive iron-sulfur protein, *J. Biol. Chem.*, 254, 333, 1979.
55. **Reem, G. H.,** *De novo* purine biosynthesis by two pathways in Burkitt lymphoma cells and in human spleen, *J. Clin. Invest.*, 51, 1058, 1972.
56. **Tsuda, M., Katunuma, N., Morris, H. P., and Weber, G.,** Purification, properties, and immunotitration of hepatoma glutamine phosphoribosylpyrophosphate amidotransferase (amidophosphoribosyltransferase, EC 2.4.2.14), *Cancer Res.*, 39, 305, 1979.
57. **Lewis, J. M. and Hartman, S. C.,** Amidophosphoribosyltransferase (chicken liver), in *Methods in Enzymology*, Vol. L1, Hoffee, P. A. and Jones, M. E., Eds., Academic Press, New York, 1978, 171.
58. **Dixon, N. E., Gazzola, C., Blakeley, R. L., and Zerner, B.,** Metal ions in enzymes using ammonia or amides, *Science*, 191, 1144, 1976.
59. **Krenitsky, T. A., Papaioannou, R., and Elion, G. B.,** Human hypoxanthine phosphoribosyltransferase. I. Purification properties, and specificity, *J. Biol. Chem.*, 244, 1263, 1969.
60. **Arnold, W. J. and Kelley, W. N.,** Human hypoxanthine-guanine phosphoribosyltransferase. Purification and subunit structure, *J. Biol. Chem.*, 246, 7398, 1971.
61. **Olsen, A. S. and Milman, G.,** Chinese hamster hypoxanthine-guanine phosphoribosyltransferase. Purification, structural, and catalytic properties, *J. Biol. Chem.*, 249, 4030, 1974.
62. **Miller, R. L. and Bieber, A. L.,** Purification and properties of inosine monophosphate:pyrophosphate phosphoribosyltransferase (EC 2.4.2.8) from brewers yeast, *Biochemistry*, 7, 1420, 1968.
63. **Kornberg, A., Lieberman, I., and Simms, E. S.,** Enzymatic synthesis of purine nucleotides, *J. Biol. Chem.*, 215, 417, 1955.
64. **Schmidt, R., Wiegand, H., and Reichert, U.,** Purification and characterization of the hypoxanthine-guanine phosphoribosyltransferase from *Saccharomyces cerevisiae*, *Eur. J. Biochem.*, 93, 355, 1979.
65. **Hochstadt, J.,** Hypoxanthine phosphoribosyltransferase and guanine phosphoribosyltransferase from enteric bacteria, in *Methods in Enzymology*, Vol. L1, Hoffee, P. A. and Jones, M. E., Eds., Academic Press, New York, 1978, 549.
66. **Miller, R. L., Ramsey, G. A., Krenitsky, T. A., and Elion, G. B.,** Guanine phosphoribosyltransferase from *Escherichia coli*, specificity and properties, *Biochemistry*, 11, 4723, 1972.
67. **Carter, C. E.,** Reaction of 6-mercaptopurine with inosine- and quanosine-5'-phosphate pyrophosphorylase purified from *E. coli*, *Biochem. Pharmacol.*, 2, 105, 1959.
68. **Gutensohn, W. and Guroff, G.,** Hypoxanthine-guanine-phosphoribosyltransferase from rat brain (purification, kinetic properties, development and distribution), *J. Neurochem.*, 19, 2139, 1972.
69. **Hughes, S. H., Wahl, G. M., and Capecchi, M. R.,** Purification and characterization of mouse hypoxanthine-guanine phosphoribosyltransferase, *J. Biol. Chem.*, 250, 120, 1975.

70. **Gutensohn, W., Huber, M., and Jahn, H.,** Facilitated purification of hypoxanthine phosphoribosyltransferase, *Hoppe-Seyler's Z. Physiol. Chem.,* 357, 1379, 1976.
71. **Natsumeda, Y., Yoshino, M., and Tsushima, K.,** Hypoxanthine-guanine phosphoribosyltransferase from rat liver. Effects of magnesium 5-phosphoribosyl 1-pyrophosphate on the chemical modification and stability of the enzyme, *Biochim. Biophys. Acta,* 483, 63, 1977.
72. **Krauss, S. W. and Milman, G.,** Characterization of cyanogen bromide peptides of hypoxanthine phosphoribosyltransferase by a micromethod, *Anal. Biochem.,* 82, 38, 1977.
73. **Friedrich, U. and Coffino, P.,** Hypoxanthine-guanine phosphoribosyltransferase with altered substrate affinity in mutant mouse lymphoma cells, *Biochim. Biophys. Acta,* 483, 70, 1977.
74. **Henderson, J. F., Brox, L. W., Kelley, W. N., Rosenbloom, F. M., and Seegmiller, J. E.,** Kinetic studies of hypoxanthine-guanine phosphoribosyltransferase, *J. Biol. Chem.,* 243, 2514, 1968.
75. **Krenitsky, T. A. and Papaioannou, R.,** Human hypoxanthine phosphoribosyltransferase. II. Kinetics and chemical modification, *J. Biol. Chem.,* 244, 1271, 1969.
76. **Olsen, A. S. and Milman, G.,** Subunit molecular weight of human hypoxanthine-guanine phosphoribosyltransferase, *J. Biol. Chem.,* 249, 4038, 1974.
77. **Muensch, H. and Yoshida, A.,** Purification and characterization of human hypoxanthine/guanine phosphoribosyltransferase, *Eur. J. Biochem.,* 76, 107, 1977.
78. **Olsen, A. S. and Milman, G.,** Human hypoxanthine phosphoribosyltransferase. Purification and properties, *Biochemistry,* 16, 2501, 1977.
79. **Holden, J. A. and Kelley, W. N.,** Human hypoxanthine-guanine phosphoribosyltransferase: evidence for tetrameric structure, *J. Biol. Chem.,* submitted for publication, 1979.
80. **Giacomello, A. and Salerno, C.,** Human hypoxanthine-guanine phosphoribosyltransferase. Steady state kinetics of the forward and reverse reactions, *J. Biol. Chem.,* 253, 6038, 1978.
81. **Gutensohn, W. and Huber, M.,** Irreversible inactivation of hypoxanthine phosphoribosyltransferase by periodate oxidized nucleotides, *Hoppe-Seyler's Z. Physiol. Chem.,* 356, 431, 1975.
82. **Gutensohn, W. and Jahn, H.,** Irreversible inhibition of hypoxanthine phosphoribosyltransferase. Further studies on the specificity of periodate-oxidized GMP, *Hoppe-Seyler's Z. Physiol. Chem.,* 358, 939, 1977.
83. **Johnson, L. A., Gordon, R. B., and Emmerson, B. T.,** Guanosine dialdehyde, an active site directed inhibitor of hypoxanthine guanine phosphoribosyltransferase, *Proc. Aust. Biochem. Soc.,* 11, (Abstr.), 27, 1978.
84. **Olsen, A. S. and Milman, G.,** Hypoxanthine phosphoribosyltransferase from Chinese hamster brain and human erythrocytes, in *Methods in Enzymology,* Vol. LI, Hoffee, P. A. and Jones, M. E., Eds., Academic Press, New York, 1978, 543.
85. **Strauss, M., Behlke, J., and Goerl, M.,** Evidence against the existence of real isozymes of hypoxanthine phosphoribosyltransferase, *Eur. J. Biochem.,* 90, 89, 1978.
86. **Johnson, G. G., Eisenberg, L. R., and Migeon, B. R.,** Human and mouse hypoxanthine-guanine phosphoribosyltransferase: dimers and tetramers, *Science,* 203, 174, 1979.
87. **Kelley, W. N. and Wyngaarden, J. B.,** The Lesch-Nyhan syndrome, in *The Metabolic Basis of Inherited Disease,* 4th ed., Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., Eds., McGraw-Hill, New York, 1978, 1011.
88. **Kelley, W. N. and Arnold, W. J.,** Human hypoxanthine-guanine phosphoribosyltransferase: studies on the normal and mutant forms of the enzyme, *Fed. Proc. Fed. Am. Soc. Exp. Biol.,* 32, 1656, 1973.
89. **Thomas, C. B., Arnold, W. J., and Kelley, W. N.,** Human adenine phosphoribosyltransferase. Purification, subunit structure, and substrate specificity, *J. Biol. Chem.,* 248, 2529, 1973.
90. **Kenimer, J. G., Young, L. G., and Groth, D. P.,** Purification and properties of rat liver adenine phosphoribosyltransferase, *Biochim. Biophys. Acta,* 384, 87, 1975.
91. **Holden, J. A., Meredith, G. S., and Kelley, W. N.,** Human adenine phosphoribosyltransferase: affinity purification, subunit structure, amino acid composition, and peptide mapping, *J. Biol. Chem.,* 254, 6951, 1979.
92. **Arnold, W. J. and Kelley, W. N.,** Adenine phosphoribosyltransferase, in *Methods in Enzymology,* Vol. LI, Hoffee, P. A. and Jones, M. E., Eds., Academic Press, New York, 1978, 568.
93. **Groth, D. P., Young, L. G., and Kenimer, J. G.,** Adenine phosphoribosyltransferase from rat liver, in *Methods in Enzymology,* Vol. LI, Hoffee, P. A. and Jones, M. E., Eds., Academic Press, New York, 1978, 574.
94. **Hochstadt, J.,** Adenine phosphoribosyltransferase from *Escherichia coli,* in *Methods in Enzymology,* Vol. LI, Hoffee, P. A. and Jones, M. E., Eds., Academic Press, New York, 1978, 558.
95. **Hori, M. and Henderson, J. F.,** Purification and properties of adenylate pyrophosphorylase from Ehrlich ascites tumor cells, *J. Biol. Chem.,* 241, 1406, 1966.
96. **Srivastava, S. K. and Beutler, E.,** Purification and kinetic studies of adenine phosphoribosyltransferase from human erythrocytes, *Arch. Biochem. Biophys.,* 142, 426, 1971.

97. **Gadd, R. E. A. and Henderson, J. F.,** Studies of the binding of phosphoribosylpyrophosphate to adenine phosphoribosyltransferase, *J. Biol. Chem.,* 245, 2979, 1970.
98. **Hori, M. and Henderson, J. F.,** Kinetic studies of adenine phosphoribosyltransferase, *J. Biol. Chem.,* 241, 3404, 1966.
99. **Krenitsky, T. A., Neil, S. M., Elion, G. B., and Hitchings, G. H.,** Adenine phosphoribosyltransferase from monkey liver. Specificity and properties, *J. Biol. Chem.,* 244, 4779, 1969.
100. **Berlin, R. D.,** Adenylate pyrophosphorylase: purification, reaction sequence, and inhibition by sodium ion, *Arch. Biochem. Biophys.,* 134, 120, 1969.
101. **Kasbekar, D. K., Nagabhushanam, A., and Greenberg, D. M.,** Purification and properties of orotic acid-decarboxylating enzymes from calf thymus, *J. Biol. Chem.,* 239, 4245, 1964.
102. **Ashihara, H.,** Orotate phosphoribosyltransferase and orotidine-5′-monophosphate decarboxylase of black gram *(Phaseolus mungo)* seedlings, *Z. Pflanzenphysiol.,* 87, 225, 1978.
103. **Yoshimoto, A., Amaya, T., Kobayashi, K., and Tomita, K.,** Orotate phosphoribosyltransferase (yeast), in *Methods in Enzymology,* Vol. L1, Hoffee, P. A. and Jones, M. E., Eds., Academic Press, New York, 1978, 69.
104. **Belser, W. L. and Wild, J. R.,** Orotate phosphoribosyltransferase: orotidylate decarboxylase *(Serratia),* in *Methods in Enzymology,* Vol. L1, Hoffee, P. A. and Jones, M. E., Eds., Academic Press, New York, 1978, 135.
105. **Silva, R. F. and Hatfield, D.,** Orotate phosphoribosyltransferase: orotidylate decarboxylase (erythrocyte), in *Methods in Enzymology,* Vol. L1, Hoffee, P. A. and Jones, M. E., Eds., Academic Press, New York, 1978, 143.
106. **Reyes, P. and Guganig, M. E.,** Studies on a pyrimidine phosphoribosyltransferase from murine leukemia P1534J. Partial purification, substrate specificity, and evidence for its existence as a bifunctional complex with orotidine 5′-phosphate decarboxylase, *J. Biol. Chem.,* 250, 5097, 1975.
107. **Lieberman, I., Kornberg, A., and Simms, E. S.,** Enzymatic synthesis of pyrimidine nucleotides. Orotidine-5′-phosphate and uridine-5′-phosphate, *J. Biol. Chem.,* 215, 403, 1955.
108. **Victor, J., Greenberg, L. B., and Sloan, D. L.,** Studies on the kinetic mechanism of orotate phosphoribosyltransferase from yeast, *J. Biol. Chem.,* 254, 2647, 1979.
109. **Rattke, W. and Krauss, G. J.,** Orotidine-5′-phosphate:pyrophosphate-phosphoribosyltransferase and orotidine-5′ phosphate:carboxy-lyase of *Euglena gracilis, Biochem. Physiol. Pflanz.,* 171, 563, 1977.
110. **Jones, M. E., Kavipurapu, P. R., and Traut, T. W.,** Orotate phosphoribosyltransferase: orotidylate decarboxylase (Ehrlich ascites cell), in *Methods in Enzymology,* Vol. L1, Hoffee, P. A. and Jones, M. E., Eds., Academic Press, New York, 1978, 155.
111. **Brown, G. K. and O'Sullivan, W. J.,** Inhibition of human erythrocyte orotidylate decarboxylase, *Biochem. Pharmacol.,* 26, 1947, 1977.
112. **Traut, T. W. and Jones, M. E.,** Kinetic and conformational studies of the orotate phosphoribosyltransferase:orotidine-5′-phosphate decarboxylase enzyme complex from mouse Ehrlich ascites cells, *J. Biol. Chem.,* 252, 8374, 1977.
113. **Brown, G. K. and O'Sullivan, W. J.,** Subunit structure of the orotate phosphoribosyltransferase-orotidylate decarboxylase complex from human erythrocytes, *Biochemistry,* 16, 3235, 1977.
114. **Reyes, P. and Intress, C.,** Coordinate behavior of orotate phosphoribosyltransferase and orotidylate decarboxylase in developing mouse liver and brain, *Life Sci.,* 22, 577, 1978.
115. **Traut, T. W. and Jones, M. E.,** Interconversion of different molecular weight forms of the orotate phosphoribosyltransferase · orotidine-5′-phosphate decarboxylase enzyme complex from mouse Ehrlich ascites cells, *J. Biol. Chem.,* 254, 1143, 1979.
116. **Reyes, P. and Sandquist, R. B.,** Purification of orotate phosphoribosyltransferase and orotidylate decarboxylase by affinity chromatography on Sepharose® dye derivatives, *Anal. Biochem.,* 88, 522, 1978.
117. **Molloy, A. and Finch, L. R.,** Uridine-5′-monophosphate pyrophosphorylase activity from *Escherichia coli, FEBS Lett.,* 5, 211, 1969.
118. **Lindsay, R. H., Tillery, C. R., and Wong Yu, M. Y.,** Conversion of the antithyroid drug 2-thiouracil to 2-thio-5′-UMP by UMP pyrophosphorylase, *Arch. Biochem. Biophys.,* 148, 466, 1972.
119. **Fast, R. and Sköld, O.,** Biochemical mechanism of uracil uptake regulation in *Escherichia coli* B. Allosteric effects on uracil phosphoribosyltransferase under stringent conditions, *J. Biol. Chem.,* 252, 7620, 1977.
120. **Natalini, P., Fioretti, E., Ruggieri, S., Vita, A., and Magni, G.,** Presence of a specific uridine 5′-monophosphate pyrophosphorylase in baker's yeast, *Experientia,* 31, 1008, 1975.
121. **Natalini, P., Ruggieri, S., Santarelli, I., Vita, A., and Magni, G.,** Baker's yeast UMP:pyrophosphate phosphoribosyltransferase. Purification, enzymatic and kinetic properties, *J. Biol. Chem.,* 254, 1558, 1979.
122. **Bressan, R. A., Murray, M. G., Gale, J. M., and Ross, C. W.,** Properties of pea seedling uracil phosphoribosyltransferase and its distribution in other plants, *Plant Physiol.,* 61, 442, 1978.

123. **Morton, D. P. and Parsons, S. M.,** Biosynthetic direction substrate kinetics and product inhibition studies on the first enzyme of histidine biosynthetis, adenosine triphosphate phosphoribosyltransferase, *Arch. Biochem. Biophys.*, 175, 677, 1976.
124. **Kleeman, J. E. and Parsons, S. M.,** Reverse direction substrate kinetics and inhibition studies on the first enzyme of histidine biosynthesis, adenosine triphosphate phosphoribosyltransferase, *Arch. Biochem. Biophys.*, 175, 687, 1976.
125. **Tebar, A. R., Ballesteros, A., and Soria, J.,** Mn^{2+} electron spin resonance studies on ATP phosphoribosyltransferase from *E. coli, Experientia,* 33, 1292, 1977.
126. **Martin, R. G.,** The first enzyme in histidine biosynthesis: the nature of feedback inhibition by histidine, *J. Biol. Chem.*, 238, 257, 1963.
127. **Sterboul, C. C., Kleeman, J. E., and Parsons, S. M.,** Purification and characterization of a mutant ATP phosphoribosyltransferase hypersensitive to histidine feedback inhibition, *Arch. Biochem. Biophys.*, 181, 632, 1977.
128. **Ames, B. N., Martin, R. G., and Garry, B. J.,** The first step of histidine biosynthesis, *J. Biol. Chem.*, 236, 2019, 1961.
129. **Voll, M. J., Appella, E., and Martin, R. G.,** Purification and composition studies of phosphoribosyl-adenosine triphosphate: pyrophosphate phosphoribosyltransferase, the first enzyme of histidine biosynthesis, *J. Biol. Chem.*, 242, 1760, 1967.
130. **Parsons, S. M. and Lipsky, M.,** Composition of the first enzyme of histidine biosynthesis isolated from wild-type and mutant operator strains of *Salmonella typhimurium, J. Bacteriol.*, 121, 485, 1975.
131. **Parsons, S. M. and Koshland, D. E., Jr.,** A rapid isolation of phosphoribosyladenosine triphosphate synthetase and comparison to native enzyme, *J. Biol. Chem.*, 249, 4104, 1974.
132. **Piszkiewicz, D., Tilley, B. E., Rand-Meir, T., and Parsons, S. M.,** Amino acid sequence of ATP phosphoribosyltransferase of *Salmonella typhimurium, Proc. Natl. Acad. Sci. U.S.A.*, 76, 1589, 1979.
133. **Tébar, A. R., Ballesteros, A., and Soria, J.,** Spin label studies of ATP phosphoribosyltransferase of *E. coli, Experientia,* 33, 1014, 1977.
134. **Tébar, A. R., Leyva, J. C., Laynez, J., and Ballesteros, A.,** The action of inhibitors (histidine and AMP) on the ATP phosphoribosyltransferase of *E. coli, Rev. Esp. Fisiol.*, 34, 159, 1978.
135. **Morton, D. P. and Parsons, S. M.,** Synergistic inhibition of ATP phosphoribosyltransferase by guanosine tetraphosphate and histidine, *Biochem. Biophys. Res. Commun.*, 74, 172, 1977.
136. **Bell, R. M., Parsons, S. M., Dubravac, S. A., Redfield, A. G., and Koshland, D. E., Jr.,** Characterization of slowly interconvertible states of phosphoribosyladenosine triphosphate synthetase dependent on temperature, substrates, and histidine, *J. Biol. Chem.*, 249, 4110, 1974.
137. **Allen, J. D. and Parsons, S. M.,** Nitrocellulose filter binding: quantitation of the histidyl-tRNA-ATP phosphoribosyltransferase complex, *Anal. Biochem.*, 92, 22, 1979.
138. **Goldberger, R. F. and Kovach, J. S.,** Regulation of histidine biosynthesis in *Salmonella typhimurium, Curr. Top. Cell. Regul.*, 5, 285, 1972.
139. **Blasi, F., Bruni, C. B., Avitabile, A., Deeley, R. G., Goldberger, R. F., and Meyers, M. M.,** Inhibition of transcription of the histidine operon *in vitro* by the first enzyme of the histidine pathway, *Proc. Natl. Acad. Sci. U. S. A.*, 70, 2692, 1973.
140. **Meyers, M., Blasi, F., Bruni, C. B., Deeley, R. G., Kovach, J. S., Levinthal, M., Mullinix, K. P., Vogel, T., and Goldberger, R. F.,** Specific binding of the first enzyme for histidine biosynthesis to the DNA of the histidine operon, *Nucl. Acids Res.*, 2, 2021, 1975.
141. **DiNocera, P. P., Avitabile, A., and Blasi, F.,** *In vitro* transcription of the *Escherichia coli* histidine operon primed by dinucleotides. Effect of the first histidine biosynthetic enzyme, *J. Biol. Chem.*, 250, 8376, 1975.
142. **Moyed, H. S. and Magasanik, B.,** The biosynthesis of the imidazole ring of histidine, *J. Biol. Chem.*, 235, 149, 1960.
143. **Bell, R. M. and Koshland, D. E., Jr.,** Allosteric properties of the first enzyme of the histidine operon, *Bioorg. Chem.*, 1, 409, 1971.
144. **Hütter, R. and DeMoss, J. A.,** Organization of the tryptophan pathway: a phylogenetic study of the fungi, *J. Bacteriol.*, 94, 1896, 1967.
145. **Largen, M., Mills, S. E., Rowe, J., and Yanofsky, C.,** Purification, subunit structure and partial amino-acid sequence of anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase from the enteric bacterium *Serratia marcescens, Eur. J. Biochem.*, 67, 31, 1976.
146. **Largen, M., Mills, S. E., Rowe, J., and Yanofsky, C.,** Purification and properties of a third form of anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase from the enterobacteriaceae, *J. Biol. Chem.*, 253, 409, 1978.
147. **Henderson, E. J., Zalkin, H., and Hwang, L. H.,** The anthranilate synthetase-anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase aggregate. Catalytic and regulatory properties of aggregated and unaggregated forms of anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase, *J. Biol. Chem.*, 245, 1424, 1970.

148. **Grieshaber, M. and Bauerle, R.,** Monomeric and dimeric forms of component II of the anthranilate synthetase-anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase complex of *Salmonella typhimurium.* Implications concerning the mode of assembly of the complex, *Biochemistry,* 13, 373, 1974.
149. **Grove, T. H. and Levy, H. R.,** Anthranilate synthase-anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase from *Salmonella typhimurium, Biochim. Biophys. Acta,* 445, 464, 1976.
150. **Grieshaber, M. and Bauerle, R.,** Structure and evolution of a bifunctional enzyme of the tryptophan operon, *Nature (London) New Biol.,* 236, 232, 1972.
151. **Robb, F., Hutchinson, M. A., and Belser, W. L.,** Anthranilate synthetase. Some physical and kinetic properties of the enzyme from *Serratia marcescens, J. Biol. Chem.,* 246, 6908, 1971.
152. **Li, S. L., Hanlon, J., and Yanofsky, C.,** Structural homology of the glutamine amidotransferase subunits of the anthranilate synthetases of *Escherichia coli, Salmonella typhimurium* and *Serratia marcescens, Nature (London),* 248, 48, 1974.
153. **Egan, A. F. and Gibson, F.,** Anthranilate synthase and anthranilate-5'-phosphoribosyl-1-pyrophosphate phosphoribosyl transferase (PR transferase) aggregate from *Aerobacter aerogenes,* in *Methods in Enzymology,* Vol. XVIIA, Tabor, H. and Tabor, C. W., Eds., Academic Press, New York, 1970, 380.
154. **Robison, P. D. and Levy, H. R.,** Metal ion requirement and tryptophan inhibition of normal and variant anthranilate synthase-anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase complexes from *Salmonella typhimurium, Biochim. Biophys. Acta,* 445, 475, 1976.
155. **Nagano, H., Zalkin, H., and Henderson, E. J.,** The anthranilate synthetase-anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase aggregate, *J. Biol. Chem.,* 245, 3810, 1970.
156. **Grove, T. H. and Levy, H. R.,** Evidence for an essential lysine residue on the phosphoribosyl transferase subunit of the anthranilate synthetase-anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase enzyme complex from *Salmonella typhimurium, Biochem. Biophys. Res. Commun.,* 86, 387, 1979.
157. **Creighton, T. E. and Yanofsky, C.,** Chorismate to tryptophan *(Escherichia coli)* — anthranilate synthetase, PR transferase, PRA isomerase, InGP synthetase, tryptophan synthetase, in *Methods in Enzymology,* Vol. XVIIA, Tabor, H. and Tabor, C. W., Eds., Academic Press, New York, 1970, 365.
158. **Goitein, R. K., Chelsky, D., and Parsons, S. M.,** Primary ^{14}C and α secondary ^{3}H substrate kinetic isotope effects for some phosphoribosyltransferases, *J. Biol. Chem.,* 253, 2963, 1978.
159. **Metzler, D. E.,** *Biochemistry. The Chemical Reactions of Living Cells,* Academic Press, New York, 1977, 302.
160. **Levitt, M.,** Conformational preferences of amino acids in globular proteins, *Biochemistry,* 17, 4277, 1978.
161. **Rossmann, M. G., Liljas, A., Brändén, C. I., and Banaszak, L. J.,** Evolutionary and structural relationships among dehydrogenases, in *The Enzymes,* Vol. XI, 3rd ed., Boyer, P. D., Ed., Academic Press, New York, 1975, 61.
162. **Rossmann, M. G. and Argos, P.,** The taxonomy of binding sites in proteins, *Mol. Cell. Biochem.,* 21, 161, 1978.
163. **Stellwagen, E.,** Use of blue dextran as a probe for the nicotinamide adenine dinucleotide domain in proteins, *Acc. Chem. Res.,* 10, 92, 1977.
164. **Ashton, A. R. and Polya, G. M.,** The specific interaction of cibacron and related dyes with cyclic nucleotide phosphodiesterase and lactate dehydrogenase, *Biochem. J.,* 175, 501, 1978.
165. **Musick, W. D. L. and Mahoney, W. A.,** manuscript in preparation, 1979.
166. **Wassarman, P. M. and Lentz, P. J., Jr.,** The interaction of tetra-iodofluorescein with dogfish muscle lactate dehydrogenase: a chemical and X-ray crystallographic study, *J. Mol. Biol.,* 60, 509, 1971.
167. **Cheshire, R. M. and Park, M. V.,** The inhibition of lactate dehydrogenase by salicylate, *Int. J. Biochem.,* 8, 637, 1977.
168. **Somerville, L. L. and Quiocho, F. A.,** The interaction of tetraiodofluorescein with creatine kinase, *Biochim. Biophys. Acta,* 481, 493, 1977.
169. **Seegmiller, J. E., Rosenbloom, F. M., and Kelley, W. N.,** Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis, *Science,* 155, 1682, 1967.
170. **Upchurch, K. S., Leyva, A., Arnold, W. J., Holmes, E. W., and Kelley, W. N.,** Hypoxanthine phosphoribosyltransferase deficiency: association of reduced catalytic activity with reduced levels of immunologically detectable enzyme protein, *Proc. Natl. Acad. Sci. U.S.A.,* 72, 4142, 1975.
171. **Ghangas, G. S. and Milman, G.,** Radioimmune determination of hypoxanthine phosphoribosyltransferase crossreacting material in erythrocytes of Lesch-Nyhan patients, *Proc. Natl. Acad. Sci. U.S.A.,* 72, 4147, 1975.
172. **Ghangas, G. S. and Milman, G.,** Hypoxanthine phosphoribosyltransferase: two-dimensional gels from normal and Lesch-Nyhan hemolyzates, *Science,* 196, 1119, 1977.

173. **Wyngaarden, J. B. and Kelley, W. N.,** Gout, in *The Metabolic Basis of Inherited Disease,* 4th ed., Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., Eds., McGraw-Hill, New York, 1978, 916.
174. **Rosenbloom, F. M., Kelley, W. N., Miller, J., Henderson, J. F., and Seegmiller, J. E.,** Inherited disorder of purine metabolism. Correlation between central nervous system dysfunction and biochemical defects, *JMA,* 202, 175, 1967.
175. **Howard, W. J., Kerson, L. A., and Appel, S. H.,** Synthesis *de novo* of purines in slices of rat brain and liver, *J. Neurochem.,* 17, 121, 1970.
176. **Kelley, W. N., Greene, M. L., Rosenbloom, F. M., Henderson J. F., and Seegmiller, J. E.,** Hypoxanthine-guanine phosphoribosyltransferase deficiency in gout, *Ann. Intern. Med.,* 70, 155, 1969.
177. **Boyd, E. M., Dolman, M., Knight, L. M., and Sheppard, E. P.,** The chronic oral toxicity of caffeine, *Can. J. Physiol. Pharmacol.,* 43, 995, 1965.
178. **Rosenbloom, F. M., Kelley, W. N., and Seegmiller, J. E.,** unpublished results, 1979.
179. **McKeran, R. O., Howell, A., Andrews, T. M., Watts, R. W. E., and Arlett, C. F.,** Observations of the growth *in vitro* of myeloid progenitor cells and fibroblasts from hemizygotes and heterozygotes for "complete" and "partial" HGPRT deficiency, and their relevance to the pathogenesis of brain damage in the Lesch-Nyhan syndrome, *J. Neurol. Sci.,* 22, 183, 1974.
180. **Emmerson, P. T. and Thompson, L.,** The spectrum of hypoxanthine-guanine phosphoribosyltransferase deficiency, *Q. J. Med.,* 42, 423, 1973.
181. **Kelley, W. N., Rosenbloom, F. M., Henderson, J. F., and Seegmiller, J. E.,** A specific enzyme defect in gout associated with overproduction of uric acid, *Proc. Natl. Acad. Sci. U.S.A.,* 57, 1735, 1967.
182. **Littlefield, J. W.,** Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants, *Science,* 145, 709, 1964.
183. **Sperling, O., Boer, P., Persky-Brosh, S., Kanarek, E., and de Vries, A.,** Altered kinetic property of erythrocyte phosphoribosylpyrophosphate synthetase in excessive purine production, *Rev. Eur. Etud. Clin. Biol.,* 17, 703, 1972.
184. **Balis, M. E., Yip, L. C., Yü, T. F., Gutman, A. B., Cox, R., and Dancis, J.,** Unstable HPRTase in subjects with abnormal urinary oxypurine excretion, in *Purine Metabolism in Man. Enzymes and Metabolic Pathways,* Vol. 41 (Part A), Sperling, O., de Vries, A., and Wyngaarden, J. B., Eds., Plenum Press, New York, 1974, 195.
185. **Arnold, W. J., Meade, J. C., and Kelley, W. N.,** Hypoxanthine-guanine phosphoribosyltransferase: characteristics of the mutant enzyme in erythrocytes from patients with the Lesch-Nyhan syndrome, *J. Clin. Invest.,* 51, 1805, 1972.
186. **Kelley, W. N. and Meade, J. C.,** Studies on hypoxanthine-guanine phosphoribosyltransferase in fibroblasts from patients with the Lesch-Nyhan syndrome, *J. Biol. Chem.,* 246, 2953, 1971.
187. **Benke, P. J. and Herrick, N.,** Azaguanine-resistance as a manifestation of a new form of metabolic overproduction of uric acid, *Am. J. Med.,* 52, 547, 1972.
188. **Sweetman, L., Hoch, M. A., Bakay, B., Borden, M., Lesh, P., and Nyhan, W. L.,** A distinct human variant of hypoxanthine-guanine phosphoribosyltransferase, *J. Pediatr.,* 92, 385, 1978.
189. **Kogut, M. D., Donnell, G. N., Nyhan, W. L., and Sweetman, L.,** Disorder of purine metabolism due to partial deficiency of hypoxanthine-guanine phosphoribosyltransferase. A study of a family, *Am. J. Med.,* 48, 148, 1970.
190. **Bakay, B., Becker, M. A., and Nyhan, W. L.,** Reaction of antibody to normal human hypoxanthine phosphoribosyltransferase with products of mutant genes, *Arch. Biochem. Biophys.,* 177, 415, 1976.
191. **Fox, I. H., Lacroix, S., Planet, G., and Moore, M.,** Partial deficiency of adenine phosphoribosyltransferase in man, *Medicine (Baltimore),* 56, 515, 1977.
192. **Van Acker, K. J., Simmonds, H. A., Potter, C., and Cameron, J. S.,** Complete deficiency of adenine phosphoribosyltransferase. Report of a family, *New Engl. J. Med.,* 297, 127, 1977.
193. **Delbarre, F., Auscher, C., Amor, B., de Gery, A., Cartier, P., and Hamet, M.,** Gout with adenine phosphoribosyltransferase deficiency, *Biomedicine,* 21, 82, 1974.
194. **Emmerson, B. T., Gordon, R. B., and Thompson, L.,** Adenine phosphoribosyltransferase deficiency: its inheritance and occurrence in a female with gout and renal disease, *Aust. N. Z. J. Med.,* 5, 440, 1975.
195. **Fox, I. H., Meade, J. C., and Kelley, W. N.,** Adenine phosphoribosyltransferase deficiency in man. Report of a second family, *Am. J. Med.,* 55, 614, 1973.
196. **Kelley, W. N., Levy, R. I., Rosenbloom, F. M., Henderson, J. F., and Seegmiller, J. E.,** Adenine phosphoribosyltransferase deficiency: a previously undescribed genetic defect in man, *J. Clin. Invest.,* 47, 2281, 1968.
197. **Delbarre, F., Auscher, C., Amor, B., and de Gery, A.,** Gout with adenine phosphoribosyl transferase deficiency, in *Purine Metabolism in Man. Enzymes and Metabolic Pathways,* Vol. 41 (Part A), Sperling, O., de Vries, A., and Wyngaarden, J. B., Eds., Plenum Press, New York, 1974, 333.

198. **Debray, H. Cartier, P., Temstet, A., and Cendron, J.,** Child's urinary lithiasis revealing a complete deficit in adenine phosphoribosyltransferase, *Pediatr. Res.,* 10, 762, 1976.
199. **Dean, B. M., Perrett, D., Simmonds, H. A., Sahota, A., and Van Acker, K. J.,** Adenine and adenosine metabolism in intact erythrocytes deficient in adenosine monophosphate-pyrophosphate phosphoribosyltransferase: a study of two families, *Clin. Sci. Mol. Med.,* 55, 407, 1978.
200. **Barratt, T. M., Simmonds, H. A., Cameron, J. S., Potter, C. F., Rose, G. A., Arkell, D. G., and Williams, D. I.,** Complete deficiency of adenine phosphoribosyltransferase: a third case presenting as renal stones in a young child, *Arch. Dis. Child.,* 54, 25, 1979.
201. **Nyhan, W. L., James, J. A., Teberg, A. J., Sweetman, L., and Nelson, L. G.,** A new disorder of purine metabolism with behavioral manifestations, *J. Pediatr.,* 74, 20, 1969.
202. **Kelley, W. N. and Smith, L. H., Jr.,** Hereditary orotic aciduria, in *The Metabolic Basis of Inherited Disease,* 4th ed., Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S., Eds., McGraw-Hill, New York, 1978, 1045.
203. **Tatibana, M. and Shigesada, K.,** Control of pyrimidine biosynthesis in mammalian tissues. V. Regulation of glutamine-dependent carbamyl phosphate synthetase: activation by 5-phosphoribosyl 1-phosphate and inhibition by uridine triphosphate, *J. Biochem. (Tokyo),* 72, 549, 1972.
204. **Worthy, T. E., Grobner, W., and Kelley, W. N.,** Hereditary orotic aciduria: evidence for a structural gene mutation, *Proc. Natl. Acad. Sci. U.S.A.,* 71, 3031, 1974.
205. **Fox, I. H. and Kelley, W. N.,** Human phosphoribosylpyrophosphate synthetase. Distribution, purification, and properties, *J. Biol. Chem.,* 246, 5739, 1971.
206. **Roth, D. G., Shelton, E., and Deuel, T. F.,** Purification and properties of phosphoribosyl pyrophosphate synthetase from rat liver, *J. Biol. Chem.,* 249, 291, 1974.
207. **Li, T. M., Mildvan, A. S., and Switzer, R. L.,** Studies of the stereochemistry and of the role of metal ions in the mechanism of phosphoribosylpyrophosphate synthetase from *Salmonella typhimurium, J. Biol. Chem.,* 253, 3918, 1978.
208. **Becker, M. A., Kostel, P. J., and Meyer, L. J.,** Human phosphoribosylpyrophosphate synthetase. Comparison of purified normal and mutant enzymes, *J. Biol. Chem.,* 250, 6822, 1975.
209. **Roth, D. G. and Deuel, T. F.,** Stability and regulation of phosphoribosylpyrophosphate synthetase from rat liver, *J. Biol. Chem.,* 249, 297, 1974.
210. **Fox, I. H. and Kelley, W. N.,** Human phosphoribosylpyrophosphate synthetase. Kinetic mechanism and end product inhibition, *J. Biol. Chem.,* 247, 2126, 1972.
211. **Becker, M. A. and Seegmiller, J. E.,** Genetic aspects of gout, *Annu. Rev. Med.,* 25, 15, 1974.
212. **Sperling, O., Boer, P., Brosh, S., Zoref, E., and de Vries, A.,** Overproduction disease in man due to enzyme feedback resistance mutation. Purine overproduction in gout due to excessive activity of mutant feedback-resistant phosphoribosylpyrophosphate synthetase, *Enzyme,* 23, 1, 1978.
213. **Becker, M. A., Meyer, L. J., Kostel, P. J., and Seegmiller, J. E.,** Increased 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase activity and gout: diversity of structural alterations in the enzyme, *J. Clin. Invest.,* 53, 4a (Abstr.), 1974.
214. **Yen, R. C. K., Adams, W. B., Lazar, C., and Becker, M. A.,** Evidence for X-linkage of human phosphoribosylpyrophosphate synthetase, *Proc. Natl. Acad. Sci. U.S.A.,* 75, 482, 1978.
215. **Dayhoff, M. O.,** *Atlas of Protein Sequence and Structure,* Vol. 5 (and subsequent supplements), National Biomedical Research Foundation, Silver Spring, Md., 1972.
216. **Musick, W. D. L. and Mahoney, W. A.,** unpublished results, Purdue University, West Layfayette, Ind. 1980.
217. **Bakay, B. and Nyhan, W. L.,** Electrophoretic properties of hypoxanthine-guanine phosphoribosyltransferase in erythrocytes of subjects with Lesch-Nyhan syndrome, *Biochem. Genet.,* 6, 139, 1972.
218. **Bakey, B. and Nyhan, W. L.,** Activation of variants of hypoxanthine-guanine phosphoribosyltransferase by the normal enzyme, *Proc. Natl. Acad. Sci. U.S.A.,* 69, 2523, 1972.