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Human Erythrocytic Purine Nucleoside Phosphorylase: Reaction with Sugar-Modified Nucleoside Substrates†

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ABSTRACT: The kinetic parameters (K_m and V_{max}) of sugar-modified analogues of inosine and guanosine have been determined with human erythrocytic purine nucleoside phosphorylase (PNP). Steric alterations at the 2′ and 3′ positions greatly lessened or abolished substrate activity. However, the 5′-deoxy- and 2′,5′-dideoxy-β-D-ribofuranosyl and the α-L-lyxosyl analogues were good substrates, indicating that the 5′-hydroxyl and the orientation of the 5′-hydroxymethyl group are not important for binding. The sugar phosphate analogue, 5-deoxyribose 1-phosphate, was synthesized from 5′-deoxyinosine with immobilized PNP, and its presence was verified by using it in the enzymic synthesis of 5′-deoxyguanosine. The adenosine versions of the 5′-modified analogues were also found to react with adenosine deaminase, albeit at <1% of V_{max}.

Human erythrocytic purine nucleoside phosphorylase (purine nucleoside:orthophosphate ribosyltransferase, PNP, EC 2.4.2.1) has been purified to homogeneity, and its physical properties and enzymatic behavior have been studied extensively (Krenitsky et al., 1968; Parks & Agarwal, 1972; Turner et al., 1971; Agarwal, K. C., et al., 1975; Agarwal et al., 1978; Stoeckler et al., 1978a,b; Zannis et al., 1978). This enzyme catalyzes the reversible phosphorolysis of ribonucleoside and 2′-deoxyribonucleoside derivatives of hypoxanthine, guanine, xanthine, and many of their analogues by the reaction

purine nucleoside + P_i ⇌ purine base + pentose 1-phosphate

Currently there is great interest in PNP for several reasons. A genetic disease has been identified that involves a specific

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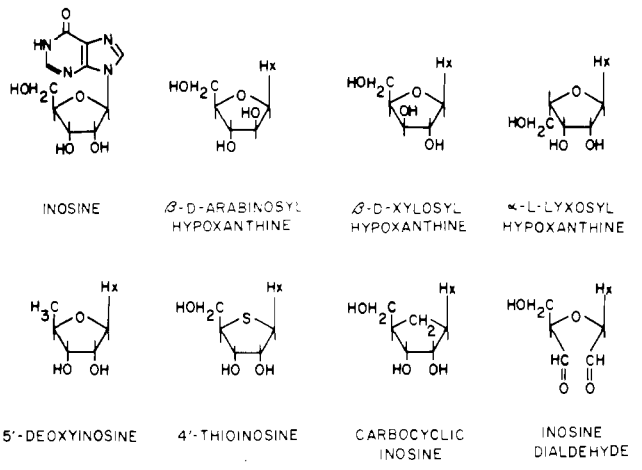

FIGURE 1: Sugar-modified analogues of inosine.

deficiency in PNP. Children with this disorder have apparently normal B lymphocytic functions and competent humoral immunity but lack T lymphocytic activity and cellular immunity (Giblett et al., 1975; Stoop et al., 1977). Further, the remarkably high activity (10–15 units/mL of cells) of PNP in human erythrocytes may degrade various nucleoside analogues of chemotherapeutic potential in transit through the blood stream to the desired site of action. Therefore, inhibitors of PNP might display immunosuppressive activity specific for T lymphocytes, as well as the capacity to potentiate the action of certain nucleoside analogues. Recent developments have indicated that immobilized human erythrocytic PNP may be a valuable catalyst for nucleoside chemists (Stoeckler & Parks, 1977; Parks et al., 1978). The enzyme may be purified to high specific activity in excellent yield and in substantial quantities from human erythrocytes, i.e., more than 1000 units/pint of human blood (Agarwal et al., 1978). The purified enzyme retains activity and is stable for many months when immobilized on CNBr-activated agarose (Stoeckler & Parks, 1977). Thus, the immobilized enzyme may be employed both for the synthesis of various pentose 1-phosphates and for the stereospecific synthesis of a variety of analogue nucleosides of chemotherapeutic interest.

Prior studies of the substrate specificity of human erythrocytic PNP have involved primarily alterations in the purine aglycon rather than the pentose, as reviewed elsewhere (Parks & Agarwal, 1972). The present report documents the activity of human erythrocytic PNP with a number of sugar-modified analogues of inosine and guanosine, some of which are presented in Figure 1. In a number of instances, sugar-modified adenosine analogues were converted to the respective inosine analogues by reaction with calf intestinal adenosine deaminase. A preliminary report of portions of this work has been presented (Parks et al., 1978).

Materials and Methods

Crystalline human erythrocytic PNP was isolated as described earlier (Agarwal & Parks, 1969; Stoeckler et al., 1978a). Some experiments were duplicated with highly purified PNP (sp act. = 65.0) that had not been crystallized and with partially purified enzyme (sp act. = 1.5). No significant differences were observed with the various preparations. Adenosine deaminase (ADA) from calf intestinal mucosa (type III, sp act. = 240), milk xanthine oxidase (type I), inosine, and guanosine were purchased from Sigma Chemical Co.; P. L. Biochemical Co. supplied 2′-deoxyinosine, 3′-deoxyadenosine, 2′,3′-dideoxyadenosine, 2′,3′-dideoxyguanosine, and 2′-*O*-methylguanosine; 2′-deoxyguanosine was purchased from Calbiochem. Samples of 5′-deoxyinosine and 5′-deoxyadenosine were kindly provided by Dr. J. G. Cory, and the latter was also prepared by Dr. S. H. Chu of this laboratory. The α-L-lyxosyladenine, a gift from Dr. L. L. Bennett, was prepared by Terra-Marine Bio-Research. The carbocyclic analogue of inosine and 5′-deoxy-5′-aminoadenosine were gifts from Drs. L. B. Townsend and S. H. Chu, respectively. The Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, provided 3′-deoxy-3′-aminoadenosine, 2′,5′-dideoxyadenosine, 4′-thioinosine, and xylosyladenine (NSC No. 18192, 95943, 90420, and 7359). Arabinosylhypoxanthine was purchased from Pfanstiehl Laboratories, Inc. Adenosine analogues were converted to their inosine counterparts by deamination with adenosine deaminase as described below. High-pressure liquid chromatography, employing reverse-phase columns under conditions described in the legend to Figure 3, demonstrated that 5′-deoxyinosine, 5′-deoxyadenosine, 2′,5′-dideoxyadenosine, α-L-lyxosylhypoxanthine, 3′-deoxyadenosine, and 3′-deoxy-3′-aminoadenosine contained less than 1% of UV-absorbing impurities. Approximately 10% of UV-absorbing impurity was present in 2′,3′-dideoxyadenosine. The contaminant could not have been responsible for the activity seen with this compound in the kinetic studies because both the deamination and phosphorolysis reactions proceeded to greater than 80% of completion. CNBr-activated Sepharose 4B was purchased from Pharmacia.

Determination of Enzyme Activities. The activity of PNP with inosine and its analogues was measured as the rate of hypoxanthine formation after its conversion to uric acid by the xanthine oxidase reaction ($\epsilon = 12.5 \times 10^3$ at 293 nm; Kalckar, 1947). Reactions were carried out at 30 °C with 0.05 M potassium phosphate, pH 7.5, and 0.02–0.04 unit of xanthine oxidase per mL. The phosphorolysis and synthesis of guanosine and its analogues were monitored directly at 258 nm with $\Delta\epsilon = \pm 5.3 \times 10^3$.[1] Earlier studies have shown that human erythrocytic PNP is subject to strong substrate activation at high concentrations of the natural nucleoside substrates (Kim et al., 1968; Agarwal & Parks, 1969; Agarwal, K. C., et al., 1975). With a number of substrate analogues definite breaks in the $1/v$ vs. $1/S$ graphs were observed (see Figure 2), and unweighted linear regression analyses were applied separately to each portion of the graph. The data in Table I represent measurements made at substrate concentrations at which no substrate activation was observed. Whenever a compound was tested, a standard assay of PNP activity with inosine was performed (Kim et al., 1968) to determine the enzyme activity on which the relative V_{max} was based. A unit of PNP activity is the amount of enzyme that converts 1 μmol of substrate to product per min in the standard assay. Inhibition of PNP activity was tested by the addition of 50 μM and greater concentrations of the analogue to a reaction mixture containing 50 μM inosine (approximately the K_m concentration), 0.05 M phosphate, and 0.02 unit of xanthine oxidase.

Adenosine deaminase activity was measured spectrophotometrically at 30 °C in 0.05 M potassium phosphate (pH 7.5) by observing the decrease in absorbance at 265 nm. The $\Delta\epsilon$ of 8.6×10^3 was assumed to apply for adenosine in the standard assay (Agarwal & Parks, 1978) as well as with analogues. In certain cases, adenosine deaminase activity was measured by the ammonia liberation procedure described earlier (Rogler-Brown et al., 1978).

[1] Unpublished observation of E. Chu of this laboratory.

Table I: Kinetic Parameters of PNP with Nucleosides Modified in the Sugar Moiety

no.	nucleoside[b]	K_m[a] (μM)	% rel V_{max}[a]
1	inosine	30[c]-46	100
2	2′-deoxyinosine	45	53
3	3′-deoxyinosine	1100 (1000-1200)	2
4	2′,3′-dideoxyinosine	440 (340-490)	~1
5	5′-deoxyinosine	31 (28-35)	45
6	2′,5′-dideoxyinosine	120 (95-143)	53 (44-61)
7	arabinosylhypoxanthine	>2000 (2000-2500)	<1
8	xylosylhypoxanthine[d]		0
9	α-L-lyxosylhypoxanthine	160 (140-180)	38 (31-45)
10	3′-amino-3′-deoxyinosine	300 (200-400)	10 (7-14)
11	5′-amino-5′-deoxyinosine[d]		0
12	4′-thioinosine	1900 (1800-2000)	<1
13	carbocyclic inosine[d]		0
14	inosine dialdehyde[d]		0
15	guanosine	32	52
16	2′-deoxyguanosine	44	42
17	5′-amino-5′-deoxyguanosine[d]		0
18	2′-*O*-methylguanosine[d]		0

[a] Range of values is given in parentheses. [b] Except where noted, all nucleosides were β-D-glycosides. [c] K_m values of 30-35 μM were observed for the reaction of inosine with PNP in freshly hemolyzed blood. [d] No activity detected with at least 5.0 units of enzyme.

Many of the inosine analogues examined were prepared from the respective adenosine analogues through deamination by reaction with calf intestinal adenosine deaminase. Since these adenosine analogues varied greatly in reactivity with ADA, it was necessary to adjust the ADA concentration and the reaction time to the particular adenosine analogue. In each case, formation of the respective inosine analogue was monitored spectrophotometrically by measuring the decrease in absorbancy at 265 nm. The adenosine analogues (1.0–5.0 mM) and 1–10 units of calf intestinal ADA were incubated in 2-mL reaction volumes for time periods necessary for complete conversion to inosine analogues, i.e., a few seconds to several hours. Dilutions of these reaction mixtures were employed in the kinetic studies with PNP without removal of the ADA. Control experiments indicated that the presence of ADA under these conditions does not affect the PNP reaction.

Immobilized Enzymes for the Synthesis of 5-Deoxyribose 1-Phosphate. For use in the synthesis of inosine analogues from adenosine analogues and in the preparation of 5-deoxyribose 1-phosphate, PNP and adenosine deaminase were immobilized on agarose by a modification of published procedures (Pharmacia Fine Chemicals, 1976). Approximately 120 units of partially purified human erythrocytic PNP (sp act. = 1.5) was dialyzed against potassium phosphate buffer (0.05 M, pH 7.5) and added to 1.0 g of CNBr-activated Sepharose in 10–20 mL of coupling buffer (0.05 M potassium phosphate, pH 8.5, and 0.5 M NaCl). The suspension was gently mixed on a rotator overnight at room temperature and then transferred to a fritted glass funnel where the gel was washed continuously with 1 L of coupling buffer. Enzymic assays indicated that ~50 units of PNP activity was bound to the Sepharose. For prolonged storage the gel was suspended in 0.05 M phosphate buffer, pH 7.5, containing 0.02% NaN_3. This preparation lost little activity on storage for 6 months at 4 °C. Columns of bound PNP have been used continuously at room temperature for periods of 6 months with retention of high activity (Stoeckler & Parks, 1977). A similar procedure was employed to attach calf intestinal adenosine deaminase (sp act. = 240) to CNBr-activated Sepharose. Usually 30–50% of the enzyme activity was recovered in bound form. The properties and behavior of these bound enzymes will be described elsewhere.

For preparation of 5-deoxyribose 1-phosphate, 5′-deoxyadenosine was first deaminated. The 5-mL reaction mixture contained bound adenosine deaminase (60 units), 5′-deoxyadenosine (0.003 M), and phosphate (0.015 M). The suspension was slowly agitated on a rotator at room temperature until no further decrease in absorbancy at 265 nm was observed, i.e., ~48 h. The filtrate from this reaction suspension was added to ~0.5 g of PNP-bound Sepharose (~25 units), which had been washed with Tris–acetate buffer (pH 7.5, 0.02 M) to remove residual phosphate buffer and sodium azide. About 0.5 unit of xanthine oxidase was added to convert the PNP reaction product, hypoxanthine, to uric acid and to drive the reaction to completion. The increase in absorbancy at 293 nm was monitored to determine the extent of reaction. After being stirred on a rotator overnight, the suspension was filtered and the inorganic phosphate was precipitated by alternating dropwise additions of barium hydroxide (saturated solution) and barium acetate (1.0 M) with maintenance of the pH at 8.0–8.5. After completion of precipitation, the barium salts were removed by centrifugation. The supernatant fluid was treated with potassium sulfate (saturated solution) to remove the slight excess of barium ions. The uric acid produced from hypoxanthine was partly removed by centrifugation upon its crystallization at 4 °C. Although uric acid did not interfere with the use of the sugar phosphate solution, it could be eliminated by adsorption on activated charcoal; however, up to 50% of the 5-deoxyribose 1-phosphate was lost during this step.

Colorimetric assays for ribose and 5-deoxyribose were performed by the ferricyanide and orcinol procedures (Ashwell, 1957). The orcinol reaction gave a color yield of only 12–25% with 5′-deoxyadenosine and 5-deoxyribose (prepared by acid hydrolysis of 5′-deoxyadenosine) when ribose was used as a standard. Therefore, since the orcinol test for ribose is less than one-tenth as sensitive as the coupled xanthine oxidase reaction for hypoxantine, it is unsuitable for the detection of 5-deoxyribose. The ferricyanide reaction proceeded equally well with ribose and 5-deoxyribose. This latter test, however, depends on a free aldehyde group and cannot be used for pentose 1-phosphates without prior hydrolysis.

Results

Activity of Sugar-Modified Analogues with PNP. Table I presents the kinetic parameters of the compounds studied. In the absence of either PNP or orthophosphate, none of the nucleosides showed a change in absorbancy at 293 nm upon incubation with xanthine oxidase. All the kinetic parameters

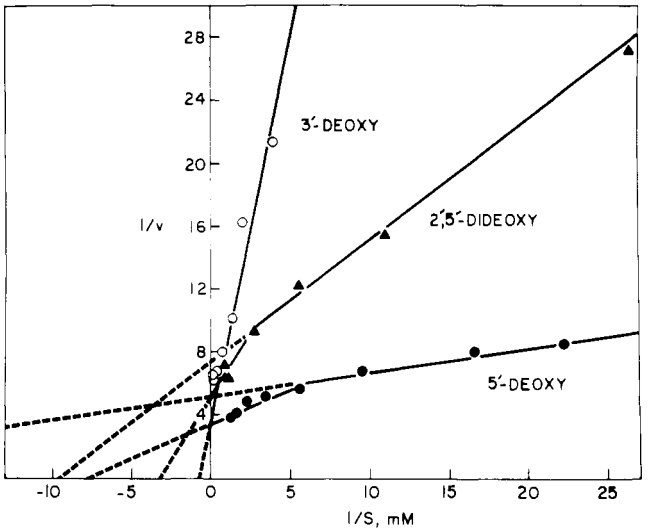

FIGURE 2: Effect of substrate concentration on the reaction velocity of PNP with 3′-deoxy-, 5′-deoxy-, and 2′,5′-dideoxyinosine. Reaction conditions were as described under Materials and Methods. The 1.0-mL reaction mixtures contained ~0.6 unit of PNP with 3′-deoxyinosine as the substrate and ~0.006 unit with the other analogues.

were obtained at relatively low substrate concentrations, i.e., in the absence of substrate activation (see below). Of the substrates studied, inosine has the best activity with PNP with a V_{max} value about twice as great as those for the 2′-deoxy, 5′-deoxy, and 2′,5′-dideoxy analogues. The V_{max} with inosine was also double that of guanosine, which, in turn, was greater than that of 2′-deoxyguanosine, in accord with earlier findings (Kim et al., 1968). Among the compounds in Table I, neither substrate binding nor reactivity was greatly affected by changes at C(5′) except when an amino group was introduced. Substrate binding was poorest in those compounds that had the lowest V_{max} values. The nucleosides that showed no substrate activity with at least 5.0 units of PNP also failed to inhibit the reaction of PNP with inosine.

Substrate Activation. An interesting characteristic of human erythrocytic PNP is the phenomenon of substrate activation observed at high concentrations of natural nucleoside substrates, e.g., inosine, 2′-deoxyinosine (Kim et al., 1968), and guanosine.[2] As shown in Figure 2, similar substrate activation occurred at high concentrations of the sugar-modified analogues 5′-deoxy- and 2′,5′-dideoxyinosine. On the other hand, with 3′-deoxyinosine (a poor substrate) no substrate activation was seen, and as concentrations were increased above 2.5 mM no further increases in reaction velocity occurred. Similar results were seen with 3′-amino-3′-deoxyinosine. The K_m values for the high substrate concentration ranges are 150 and 290 μM for 5′-deoxy- and 2′,5′-dideoxyinosine, respectively. Because of limited supplies of this compound, the 2′,5′-dideoxy analogue was only tested once at high concentrations.

Synthesis of 5-Deoxyribose 1-Phosphate. As described above, 5′-deoxyadenosine was deaminated with ADA and cleaved by reaction with PNP. After P_i and uric acid were removed from the resulting solution, the PNP reaction was employed to demonstrate the presence of 5-deoxyribose 1-phosphate. Addition of the sugar phosphate to guanine in the presence of PNP resulted in a spectral change like that seen during guanosine synthesis. Analysis of the product by high-pressure liquid chromatography (Figure 3) showed the

[2] J. D. Stoeckler (unpublished observation).

Table II: Kinetic Parameters of Calf Spleen ADA with Nucleosides Modified in the Sugar Moiety

nucleoside	K_m[a] (μM)	% rel V_{max}[a,b]
adenosine[c]	35	100
2′-deoxyadenosine[c]	22	93
5′-deoxyadenosine	330 (320–330)	<1
2′,5′-dideoxyadenosine	590 (560–620)	<1
α-L-lyxosyladenine	>2000	<1

[a] Range of values is given in parentheses. [b] The 100% activity was based on a standard assay as described under Materials and Methods. [c] Literature values (Agarwal, R. P., et al., 1975).

5′-deoxyguanosine to have a retention time distinct from that of guanosine.

Substrate Activity with Adenosine Deaminase. The inosine analogues no. 3–11 of Table I were produced from the respective adenosine analogues, which were not reactive with PNP. When the adenosine analogues were incubated with calf intestinal adenosine deaminase, it was observed that those modified at C(5′) were deaminated to completion but at extremely slow rates. The kinetic parameters for 5′-deoxy- and 2′,5′-dideoxyadenosine and α-L-lyxosyladenine, given in Table II, were obtained by spectrophotometric assays employing 6–11 units of the adenosine deaminase. Ammonia liberation assays with the 5′-deoxy and 2′,5′-dideoxy analogues demonstrated that these compounds were also deaminated slowly by human erythrocytic adenosine deaminase.

Discussion

From the above studies and others, a number of conclusions may be drawn about the significance of various components of the carbohydrate moiety to both the reaction velocity and the binding of the nucleoside substrate to the reactive site of PNP. It has long been recognized that the 2′ carbon of the pentose plays a key role. For example, the K_m values of inosine and 2′-deoxyinosine are similar, but the reaction velocity of inosine is about twofold greater. Since PNP reacts via an S_N2 mechanism with a Walden inversion at C(1′) (Tarr, 1958; Friedkin & Kalckar, 1961), it may be proposed that the hydroxyl on C(2′) contributes to the electrophilic character of C(1′). Furthermore, steric factors involving C(2′) play a crucial role. Although arabinosylhypoxanthine (Table I, Figure 1) has definite substrate activity, it has a very high K_m (2.0 mM) and low V_{max}. Also, it was not possible to detect substrate activity with 2′-*O*-methylguanosine. Even more drastic effects are seen with alterations at C(3′). If the 3′-hydroxyl group is replaced by an amino group, distinct substrate activity is retained; i.e., the reaction velocity is ~10% of that with inosine and the K_m is about sixfold higher. Furthermore, if the hydroxyl group is removed (as in 3′-deoxyinosine and 2′,3′-dideoxyinosine), marked elevations in the K_m values are observed and the V_{max} values are ~1% that of inosine. The fact that no substrate reactivity was detected with xylosylhypoxanthine supports a crucial role for steric factors involving C(3′). Modifications of C(4′) have been studied with two compounds, 4′-thioinosine and carbocyclic inosine, in which the ring oxygen has been replaced by a sulfur atom or a methylene group, respectively. With both compounds, substrate activity was abolished or drastically impaired. Earlier it was reported that carbocyclic inosine does not inhibit PNP from H. Ep. no. 2 cells (Bennett et al., 1968).

Especially intriguing are findings that involve C(5′). It is established that various substitutions of C(5′), such as phosphate groups, abolish substrate activity. Thus, it is not surprising that no activity is observed with a positively charged

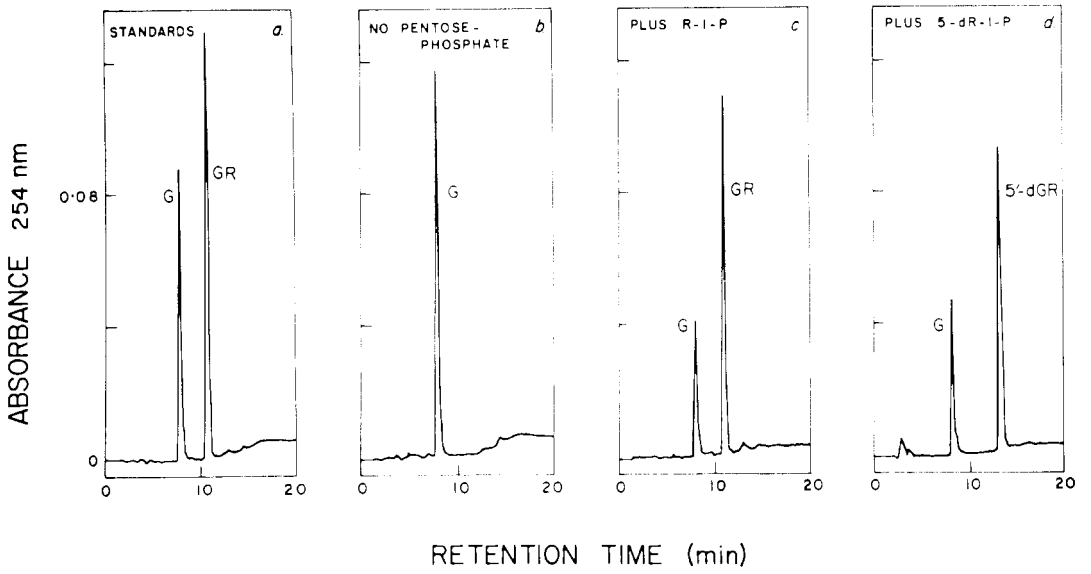

FIGURE 3: PNP-catalyzed synthesis of 5′-deoxyguanosine from 5-deoxyribose 1-phosphate and guanine. The reaction mixtures contained 70 μM guanine, ~40 μM ribose 1-phosphate or 5-deoxyribose 1-phosphate, 0.04 unit of PNP, and 0.01 M Tris-HCl, pH 7.5, in 0.5 mL. After incubation at room temperature for 6 h, 100-μL aliquots were subjected to reverse-phase high-pressure liquid chromatography on a Varian 4200 analyzer employing a Waters μBondapak C_{18} column. Elution was effected by a linear MeOH gradient (0–20% in 11 min; isocratic 20% for 4 min) in 0.01 M potassium phosphate, pH 5.5. The flow rate was 1.0 mL/min. (a) Standard solution containing 50 μM each of guanine (G) and guanosine (GR). (b) Pentose phosphate omitted from the reaction mixture. (c) Reaction with ribose 1-phosphate. (d) Reaction with 5-deoxyribose 1-phosphate.

amino group on C(5′). If the hydroxyl group is replaced by a hydrogen atom, however, as in 5′-deoxyinosine and 2′,5′-dideoxyinosine, the kinetic parameters compare favorably with those of the natural substrates. Unexpectedly, excellent substrate activity was observed with α-L-lyxosylhypoxanthine. As shown in Figure 1, this analogue may assume a configuration that closely resembles that of inosine, but with the 5′-carbon atom in a position cis rather than trans to the hydroxyl groups on C(2′) and C(3′). Thus, it appears that there is no specific binding of the enzyme to the 5′-carbon atom or its hydroxyl group since drastic steric alteration of this carbon atom has little influence on enzymic reactivity. Bennett & Hill (1975) likewise found α-L-lyxosyladenine to be a good substrate of adenosine kinase.

It has been reported by Jordan & Wu (1978) that 5′-deoxyinosine inhibits human erythrocytic PNP but does not itself undergo cleavage. This discrepancy with the present findings is difficult to explain. In the present study, production of hypoxanthine from 5′-deoxyinosine was readily detected after its conversion to uric acid. Furthermore, the production of 5-deoxyribose 1-phosphate has been demonstrated by employing it in the enzymatic synthesis of 5′-deoxyguanosine (see Figure 3.). Jordan & Wu also observed an approximately 2.5-fold higher K_m value for guanosine (83 μM) than that presented here. They studied the nucleoside substrate at high concentrations, where substrate activation occurs. As shown in Figure 2 and earlier (Agarwal & Parks, 1969), the K_m value for the substrate activation range is 3–5 times higher than that obtained at low concentrations.

Although there have been brief reports of the interactions of various sugar-modified nucleoside analogues with PNP's from several sources, there have been no detailed analyses of the kinetic parameters of this class of analogues with human erythrocytic PNP. Recently, publications have appeared on the purification and various properties of PNP's from a wide range of tissues and species, i.e., microbial (Jensen & Nygaard, 1975; Doskočil & Holý, 1977), avian (Fustě & Bozal, 1975; Murakami & Tsushima, 1976), and mammalian (Agarwal et al., 1973; Edwards et al., 1973; Moyer & Fisher, 1976; Milman et al., 1976; Lewis & Glantz, 1976a,b; Stoeckler et al., 1978a,b). These enzymes differ significantly in substrate specificities and physical structures. Therefore, since many of the nucleoside analogues under consideration in this paper and elsewhere have potential as chemotherapeutic or immunosuppressive agents, it is important to appreciate that one may not be able to predict the reactivity of such analogues with human PNP on the basis of studies performed with enzymes from other species.

Recent evidence indicates that human T lymphocytes, thymus, and spleen have relatively high activities of both PNP and deoxyguanosine kinase (Carson et al., 1977; Borgers et al., 1977). It has been proposed that PNP protects these tissues by preventing accumulation of toxic levels of dGTP from deoxyguanosine (Cohen et al., 1978; Gudas et al., 1978). Presumably, B lymphocytes, which have very low PNP activity (Borgers et al., 1977), are less susceptible to exposure to deoxyguanosine. Therefore, PNP may be an important target for the design of cytotoxic or immunosuppressive agents with specificity for cellular immunity. A nucleoside analogue with good reactivity with deoxyguanosine kinase but low activity for PNP might form cytotoxic analogue nucleotides in T lymphocytes without prior degradation by PNP. Also, if potent inhibitors of PNP could be identified and coadministered with analogues of deoxyguanosine, e.g., β-D-2′-deoxythioguanosine, enhanced intracellular formation of analogue deoxynucleotides, e.g., deoxythioGTP, might be achieved. The most potent PNP inhibitor reported to date, the inosine analogue formycin B, has a relatively high K_i value (1×10^{-4} M) but is capable of inhibiting the phosphorolysis of 6-thioinosine in intact erythrocytes (Sheen et al., 1968). It is hoped that extension of the above studies will define modifications in the carbohydrate moiety of nucleosides that will lead to the identification of more potent inhibitors. As noted elsewhere (Parks et al., 1978), purified PNP bound to a solid support such as agarose could also prove of value in the synthesis of promising analogues. Since large quantities of this enzyme are readily purified from human erythrocytes and high activity may be bound in a stable form, i.e., ~50 units/g of Sepharose, even sugar-modified

nucleosides that display low V_{max} values with PNP may be converted to the respective pentose 1-phosphates through the use of prolonged incubation times. These novel pentose 1-phosphates may be used for the synthesis of new families of nucleosides through prolonged incubation with agarose-bound PNP and base analogues such as 6-thioguanine, 6-seleno-guanine, and 8-azaguanine.

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