KOR. JOUR. MICROBIOL. July, 1991 p. 172-178 Vol. 29, No. 3

Partial Purification and Characterization of Purine Nucleoside Phosphorylase in *Saccharomyces cerevisiae**

Hye-Seon Choi
Department of Microbiology, Ulsan University, Ulsan 680-749, Korea

*Saccharomyces cerevisiae*에서 PNP의 부분 정제와 특성

최혜선
울산대학교 미생물학과

ABSTRACT: Intracellular purine nucleoside phosphorylase (PNP) from *Saccharomyces cerevisiae* was partially purified using ammonium sulfate fractionation, heat treatment, a DEAE-Sephadex A-50 anion exchange chromatography and a Sephadex G-100 gel filtration chromatography. The enzyme was purified 20 fold with 3% recovery. The stability of enzyme was kept by addition of inosine and dithiothreitol. The pH optimum was found to be from 6.3 to 7.3. PNP was sensitive to 10 mM of Hg^{2+}, Cu^{2+}, and was inactivated completely by 2 mM of *p*-chloromercuribenzoate and 5,5'-dithiobis (2-nitrobenzoate). The enzyme was capable of catalyzing the phosphorolysis of inosine, deoxyinosine, guanosine, deoxyguanosine and adenosine.

KEY WORDS □ Purine nucleoside phosphorylase, *Saccharomyces cerevisiae*, phosphorolysis

INTRODUCTION

The splitting of the glycosidic bond of a nucleoside is the critical reaction in nucleoside catabolism. The nucleoside structure, as it occurs in nucleotides and nucleic acids, is broken by this reaction. It generates purine or pyrimidine base and the corresponding pentose sugar. Two different reactions for nucleoside cleavage have been found in nature (Vogels and Drift, 1976). One is a reversible phosphorolytic cleavage of the glycosidic bond catalyzed by a nucleoside phosphorylase. The other is an irreversible hydrolytic cleavage. Three main types of nucleoside phosphorylases have been found: (1) purine nucleoside phosphorylase (Stoeckler, 1984), (2) thymidine phosphorylases (Schwartz, 1978) and (3) uridine phosphorylases (Krenitsky, 1976).

Purine nucleoside phosphorylase (PNP) catalyzes the following reaction:

purine nucleoside + phosphate $\rightleftharpoons$ purine + ribose 1−phosphate

The enzymatic degradation of purine nucleoside phosphorylase was first described by Kalckar in 1945. The equilibrium was shown to greatly favor synthesis of the nucleoside (Kalckar, 1947). The natural purine bases, guanine and hypoxanthine work as substrates. In contrast, adenine nucleosides are very poor substrates for PNP from most sources. The sugar moiety may be ribose or 2-deoxyribose.

Synthesis of purine nucleoside have been carried out with PNP from both microbial and mammalian sources. PNP was found in most mammalian tissues and other microorganisms such as *Salmonella* (Jensen and Nygaard, 1975), *Bacillus* (Gilpin and Sadoff, 1971; Jensen, 1978), *Saccharomyces* (Heppel and Hilmoe, 1952) and *Physarium*, whereas it was not detected in some microorganisms such as *Neisseria*, *Azotobacter*, *Lactobacillus* and *Penicillium* (Hammer-Jespersen, 1983). The enzyme from a variety of biological sources have been investigated and considerable variations in their properties have been found (Carlson and Fischer, 1979; Choi *et al.*, 1986; Ghanges and Rheem, 1979; Glantz and Lewis, 1978; Ikezawa *et al.*, 1978; Kazmers *et al.*, 1980; Krenitsky *et al.*, 1981; Lewis and Lowry, 1979; McRoberts and Martin, 1980; Milman, 1978; Moyer and Fischer, 1976; Mura *et al.*, 1978; Osborne, 1980; Robertson and Hoffee, 1973; Stoeckler *et al.*, 1978; 1980; 1982; Umemura *et al.*, 1982; Wiginton *et al.*, 1980; Zannis *et al.*, 1978).

*이 논문은 1990년도 문교부 지원 한국학술진흥재단의 지방대육성 학술연구조성비에 의하여 연구되었음.

On the other hand, it was reported that *Saccharomyces cerevisiae* contains PNP in moderate concentration (Heppel and Hilmoe, 1952) and it appears to play an important role in the nucleoside metabolism of these cells. In the present study, we have attempted to purify and characterize PNP from *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Purine Nucleoside Phosphorylse Assay

The assay used in these studies was the coupled xanthine oxidase method (Kalckar, 1947) which was based on the measurement of the increase in absorbance at 293 nm due to the formation of uric acid. Spectrophotometer (Kontron, UVKON 860) equipped with RS 232C-interface was used. The reaction mixture for standard assay contained, in a final volume of 1 ml, 50 mM Tris, pH 7.3, 1 mM inosine, 10 mM potassium phosphate, 0.02 μM unit of xanthine oxidase and an appropriate amount of PNP. All reaction mixtures except PNP were preincubated at 30C for about 3 min to remove any trace of hypoxanthine or xanthine as a contaminant in the inosine. The reaction was started by the addition of PNP and continued for about 3 min to get the linear region. One unit of PNP is defined as that amount of enzyme which gives an optical density increase of 12.5 per min at 293 nm under standard assay conditions.

Measurement of Protein Concentration

Protein concentrations were measured by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard or the method in which the absorbances at 280 and 260 nm were multiplied by factors of 1.55 and 0.76, respectively and subtracted to obtain the protein concentration in mg per ml.

Enzyme Purification

Baker's Yeast commercially available from Sigma Chemical Co. was used and all purification steps were carried out at 4℃. Thirty grams of Baker's Yeast were dissolved in 130 ml of 10 mM Tris, pH 7.3 containing 1 mM dithiothreitol (DTT) and 1 mM EDTA, and immediately disrupted for 3 min with a pulse in a ice-chilled bead beater (Biospec Products) with 0.5 mm of glass beads. The homogenate was clarified by centrifugation at 10,000×g for 40 min. The supernatant was brought to 35% saturation by adding solid ammonium sulfate (19.4 g/100 ml of supernatant) and stirred for 30 min. The solution was centrifuged at 10,000×g for 40 min and the pellet was discarded. More ammonium sulfate (18.4 g/100 ml of supernatant) was added to bring the concentration to 65% saturation and the pH of the solution was 5.9. Two hours later, the precipitate was collected by centrifugation at 10,000×g for 40 min and dissolved in 10 mM Tris, pH 7.3. The enzyme from ammonium sulfate fraction was dialyzed overnight against 4 l of 10 mM Tris, pH 7.3 with two changes. The dialysate was treated at 50℃ for 10 min with shaking and clarified by centrifugation at 10,000×g for 30 min. The clarified enzyme solution was applied to a DEAE-Sephadex A-50 column (3×18 cm) which had been equilibrated with 10 mM Tris, pH 7.2. The column was washed with 500 ml of the equilibration buffer until the absorbance of the eluent decreased to around 0.1 at 280 nm. Then a linear gradient was started with 10 mM Tris, pH 7.2 varing from 0 to 0.8 M NaCl in a total volume of 320 ml. The fraction size was 10 ml. Active fractions were pooled and concentrated by adding solid ammonium sulfate (47.6 g/100 ml of solution) to a saturation of 75%. The precipitate in the ammonium sulfate suspension was collected by centrifugation and dissolved in a small volume of 10 mM Tris, pH 7.2. The portion of enzyme (1/3) was loaded directly on a Sephadex G-100 column (3×42 cm) equilibrated with 20 mM Tris, pH 7.2. The column was washed with the equilibration buffer and 1 ml fractions were collected. The flow rate was 50 ml/hr. The pooled enzyme solution was concentrated by lyophilization. All the preparation which was used for characterization was obtained by several times of repeated procedures.

RESULTS AND DISCUSSION

Enzyme Purification

In ordr to localize the PNP activity, *Saccharomyces cerevisiae* cells were grown and fractionated. The enzyme was found in the disrupted intracellular materials, whereas no activity was measured in extracellular medium. Since PNP activity was also detected in intracellular preparations of lyophilized dry powder of *Saccharomyces cerevisiae*, commercially available Baker's Yeast was used for further studies.

The results are shown in Table 1. The enzyme was purified 20 fold with 3% recovery. Ammonium sulfate precipitation and heat treatment were effective to remove turbid materials and deep colors. PNP was bound on a DEAE-Sephadex A-50 column and no activity was found in the washing steps, as shown in Fig. 1. The enzyme was eluted with buffer containing 0.25-0.45 M NaCl concentration with a single peak. The major portion of PNP was subjected to a gel filtration column. The enzyme was eluted as a single peak as shown in Fig. 2.

Stability of Enzyme

The purified preparations of PNP from most mammalian tissues and other microbial origins were reported to be reasonably stable (Stoeckler *et al.*, 1978; Krenitsky *et al.*, 1981; Robertson and

Table 1. *Purification of purine nucleoside phosphorylase from Saccharomyces cerevisiae*

Step	Crude Extracts	$(NH_4)_2SO_4$ fractionation	Heat Treatment	DEAE-Sephadex A-50	Sephadex G-100 (1/3)
Total Activity (U)	35.9	17.7	14	4.8	0.35
Total Protein (mg)	9667	1128	378	115	4.8
Specific Activity (U/mg)	0.0037	0.016	0.037	0.042	0.073
Recovery (%)	100	49	39	13	3
Purification (fold)	1	4.3	10	11.4	19.7

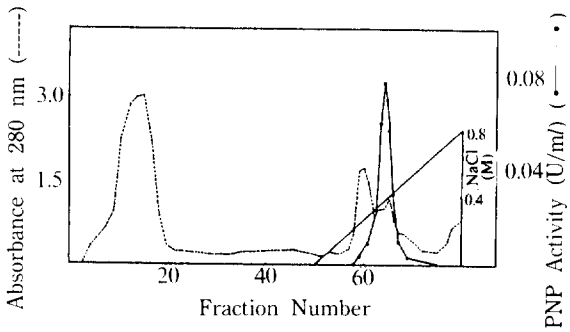

Fig. 1. *Elution profile of purine nucleoside phosphorylase from anion exchange column, DEAE-Sephadex A-50.*
The heat treated and clarified enzyme solution was applied and the column was washed and eluted as described in Materials and Methods. A_{280} for protein concentration (------); purine nucleoside phosphorylase activity (——).

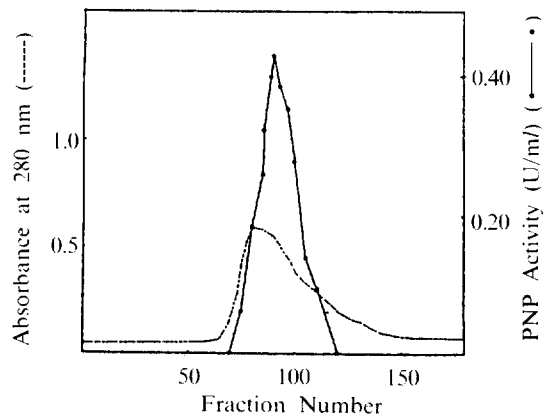

Fig. 2. *Elution profile of purine nucleoside phosphorylase from gel filtration column, Sephadex G-100.*
The pooled fractions from DEAE-Sephadex A-50 was treated with 75% ammonium sulfate. The portion of the precipitate was applied and the column was eluted as described in Materials and Methods. A_{280} for protein concentration (------); purine nucleoside phosphorylase activity (——).

Hoffee, 1973). However, PNP from *S. cerevisiae* was a labile enzyme as suggested by the low recovery during purification. Especially after the step of DEAE-Sephadex A-50 column chromatography, the activity of the enzyme decreased drastically. Some stabilizing component might be removed by an anion exchange column. The enzyme may be stored with little loss of activity as a lyophilized powder or as a suspension in 75% saturated ammonium sulfate. However, freezing and thawing in a dilute solution of 10 mM Tris, pH 7.3 resulted in the inactivation of the enzyme with about 50% loss of activity. In order to study the effect of protection from inactivation of the enzyme, PNP was treated with substrate, inosine or thiol-protective reagent, DTT. As seen in Fig. 3, the activity decayed within a few days if the enzyme was stored in 10 mM Tris, pH 7.3, whereas incubation with 10 mM DTT and 1 mM inosine have resulted in no loss of activity. The binding of substrate appeared to stabilize the enzyme and the presence of sulfhydryl groups seems to play an essential role for PNP. Treatment of the enzyme with inosine alone showed better protection than with DTT alone. It will be examined further whether the inhibition of the enzyme by sulfhydryl reagents could be overcome by substrates and (or) thiol protective reagents. It was reported that PNP from *Bacillus subtilis* and *Salmonella typhimurium* were protected by some of the substrates from inactivation by a sulfhydryl reagent, *p*-chloromercuribenzoate (*p*-

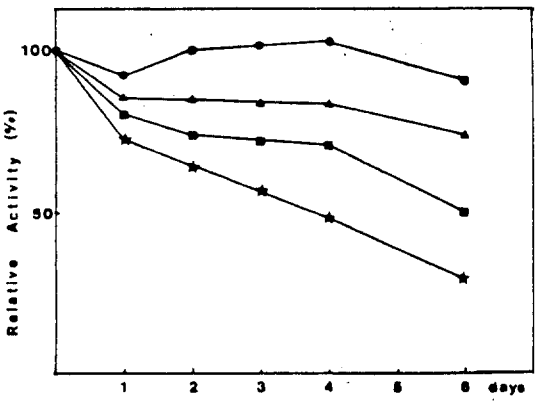

Fig. 3. *Protection of purine nucleoside phosphorylase activities from inactivation in the presence of inosine or (and) DTT. The enzyme from a gel filtration column (specific activity, 0.073 U/mg) was treated with or without inosine or (and) DTT. The reaction mixture was put at 4°C and then aliquots were collected at the indicated time points to determine the enzyme activity.* control (★); inosine (▲); DTT (■); inosine and DTT (●).

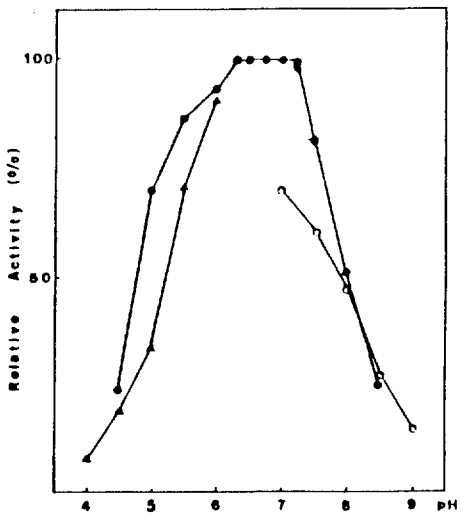

Fig. 4. *Effect of pH on purine nucleoside phosphorylase activity. The reaction mixture contained, in a final volume of 1 ml, 1 mM inosine, 25 mM potassium phosphate, 0.02 μM unit of xanthine oxidase and an appropriate buffer. After 3 min of preincubation at 30°C, the enzyme from a Sephadex G-100 column (specific activity, 0.073 U/mg) was added into the reaction mixture. The buffers of 200 mM sodium acetate (▲), 25 mM potassium phosphate (●) and 200 mM Tris (○) were used for pH 4.0-6.0, pH 4.5-8.5 and pH 7.0-9.0, respectively.*

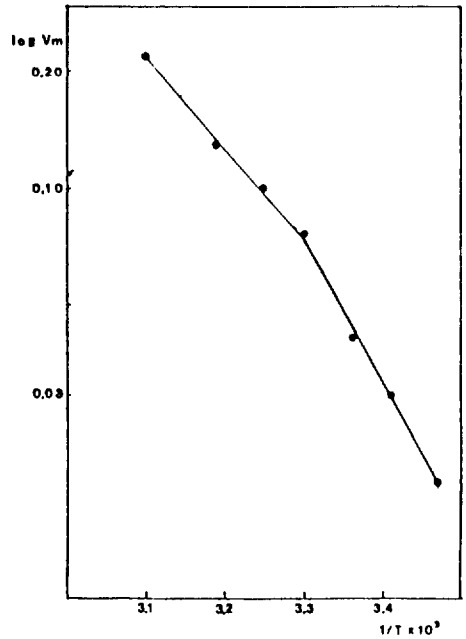

Fig. 5. *Effect of temperature on purine nucleoside phosphorylase activity.*
The reaction mixture as described in Materials and Methods was preincubated for 3 min and the reaction was started by adding the enzyme (specific activity, 0.073 U/mg). Each of the mixture ws incubated in the temperature controlled cell. Activation energy, E_a was calculated to be 10.61 and 16.94 kcal · mol^{-1} from 30-50°C and 15-30°C, respectively.

CMB) (Jensen, 1978; Jensen and Nygaard, 1978).

Effect of pH

The activity of the purified PNP was determined as a function of pH as shown in Fig. 4. A pH optimum range of about 6.3 to 7.3 was observed. In order to study whether the enzyme was inactivated irreversibly at low or high pH, PNP was preincubated at pH 4.5-8.5 for 5 min. No effect on the activity was observed under standard assay conditions (Data not shown). Thus, the decline in activity between pH 4.5 and 6.3 and between 7.3 and 8.5 must result from the formation of an improper ionic form of the enzyme and (or) substrate. The values of Km and Vm of PNP over various pH values would give an information of ionization of enzyme-substrate complex and free enzyme. It is deserving of further detailed study. Previous reports on PNP from *Escherichia coli* and *Salmonella typhimurium* showed a pH optimum of 7.0-7.5 (Krenitsky *et al.*, 1981) and 7.5 (Robertson and Hoffee, 1973), respectively. The pH chosen for kinetic and other

Table 2. *Effects of various substances on the activity of purine nucleoside phosphorylase from Saccharomyces cerevisiae*

Substance	Concentration (mM)	Remaining Activity (%)
Control		100
$MnCl_2$	10	100
$MgSO_4$	10	93
$CoCl_2$	10	84
$CaCl_2$	10	86
$ZnCl_2$	10	64
KCl	10	86
NaCl	10	86
EDTA	10	79
$HgCl_2$	10	14
$CuSO_4$	10	7
p-CMB	2	0
DTNB	2	0

Measurements were carried out in the standard assay conditions after 5 min preincubation of purified PNP with various effectors

studies was 7.3.

Effect of Temperature

In order to study the effect of temperature on the stability of enzyme, the activity of enzyme was measured at 37, 42, 50 and 60℃. After 15 min of incubation, the activities at the above conditions decreased drastically (Data not shown). However, when the initial velocities were obtained at various temperatures between 15 and 50℃, the activities of purified enzyme increased as the temperature went up. As shown in Fig. 5, a plot of log (velocity) against 1/T gave a sharp change in slope at 30℃, indicating that the enzyme occurred in two interconvertible forms with different activation energies. No denaturation of the enzyme was found under the assayed temperature range. The activation energy for the reaction was calculated from the Arrhenius equation (Segal, 1975) and to be 10.61 and 16.94 Kcal · mol^{-1} from 30 to 50℃ and from 15 to 30℃, respectively.

Effects of Metal Ion and Thiol Reagents

The effect of various metal ions at a concentration of 10 mM on the purified PNP is shown in Table 2. The enzyme was very sensitive to Hg^{2+}, Cu^{2+} and Zn^{2+} decreased the enzyme activity. Other metal ions such as Ca^{2+}, Mg^{2+}, Co^{2+}, Na^{+}, K^{+} and EDTA slightly decreased the enzyme activity. However, the reagents which reacts with thiol groups such as *p*-CMB and 5,5'-dithiobis(2-nitrobenzoate) (DTNB) at 2 mM of concentration completely inactivated the enzyme.

Table 3. *Substrate specificity of purine nucleoside phosphorylase from Saccharomyces cerevisiae*

Substrate	Relative Activity (%)
Inosine	100
Deoxyinosine	140
Guanosine	54
Deoxyguanosine	54

Enzyme activities were measured at the saturating concentration of each substrate under the standard assay conditions. The phosphorolysis of guanosine and deoxyguanosine was measured by the decrease of absorbance at 257 nm with ΔE of 5.0×10^{3} M^{-1} cm^{-1}.

Substrate Specificity

The enzyme activity towards several nucleoside was studied to elucidate the identification of the enzyme. As shown in Table 3, the enzyme was capable of catalyzing the phosphorolysis of inosine, deoxyinosine, guanosine and deoxyguanosine. Deoxyinosine showed the maximum activity. The activity ratio of 0.71 and 1.0 were found for inosine to deoxyinosine and guanosine to deoxyguanosine, respectively. In the coupled assay with xanthine oxidase to produce 2,8-dihydroxyadenine at 305 nm, adenosine also served as a substrate. However, it was not examined whether a single enzyme acts on all these nucleosides or the enzyme preparation consists of a mixture of several enzymes, each specific for a nucleoside. That question should be examined carefully in next studies. The phosphorolysis of inosine, deoxyinosine, guanosine, deoxyguanosine and adenosine was also demonstrated in PNP from *Salmonella typhimurium* (Robertson and Hoffee, 1973) and *Bacillus subtilis* (Jensen, 1978). However, adenosine is known to be a poor substrate in human erythrocytic PNP (Steockler, 1984).

적 요

*Saccharomyces cerevisiae*의 세포내 효소인 purine nucleoside phosphorylase를 ammonium sulfate fractionation, 열처리, DEAE-Sephadex A-50 음이온 교환 크로마토그래피와 Sephadex G-100겔 여과 크로마토그래피를 사용하여 부분적으로 정제하였다. 상기 효소는 20배로 정제되었고 회수율은 약 3% 였으며 효소의 안정도는 기질인 inosine과 DTT를 함께 넣어주었을 때 증가되었다. 효소의 최적 반응 pH는 6.3에서 7.3이었고 10 mM의 Hg^{+2}, Cu^{+2}에 의해 활성도가 감소되었으며 2 mM의 *p*-CMB나 DTNB 처리시 효소의 활성이 완전히 저해 되었다. 기질로서 inosine, deoxyinosine, guanosine, deoxyguanosine과 adenosine을 사용하여 효소와 반응시켰을 때 공히 인산 가수분해 되었다.

ACKNOWLEDGEMENT

This work was supported by a research grant from the Ministry of Education, the Rupublic of Korea, 1990.

REFERENCES

1. **Carlson, J.D. and A.G. Fischer,** 1979. Characterization of the active site of homogenious thyroid purine nucleoside phosphorylase. *Biochim. Biophys. Acta,* **571**, 21-28.
2. **Carlson, J.D. and A.G. Fischer,** 1979. Thyroid purine nucleoside phosphorylase II; Kinetic model by alternate substrate and inhibition studies. *Biochim. Biophys. Acta,* **566**, 259-265.
3. **Choi, H.-S., Stoeckler, J.D. and R.F. Parks, Jr.,** 1986. 5-Iodoribose 1-Phosphate, an analog of ribose 1-phosphate. *J. Biol. Chem.,* **261**, 599-607.
4. **Gilpin, R.W. and H.L. Sadoff,** 1971. Physical and catalytic properties of the purine nucleoside phosphorylases from cells and spores of *Bacillus cereus*. *J. Biol. Chem.,* **246**, 1475-1480.
5. **Ghangas, G. and G.H. Rheem,** 1979. Characterization of the subunit structure of human placental nucleoside phosphorylase by immunochemistry. *J. Biol. Chem.,* **254**, 4233-4237.
6. **Glantz, M.D. and A.S. Lewis,** 1978. Purine nucleoside phosphorylase from rabbit liver. In: Methods in enzymology, Vol. 51(Hoffee, P.A. and M.E. Jones, eds.) pp. 524-530. Academic Press, London.
7. **Hammer-Jespersen, K.,** 1983. Nucleoside catabolism. In: Metabolism of nucleotides, nucleosides and nucleobases in microorganisms (Munch-Petersen, A., ed.) pp. 203-258. Academic Press, London.
8. **Heppel, L.A. and R.J. Hilmoe,** 1952. Phosphorolysis and hydrolysis of purine ribosides by enzymes from yeast. *J. Biol. Chem.,* **198**, 683-694.
9. **Ikezawa, Z., T. Nishino, K. Murakami and K. Tsushima,** 1978. Purine nucleoside phosphorylase from bovine liver. *Comp. Biochem. Physiol.,* **60B**, 111-116.
10. **Jensen, K.F. and P. Nygaard,** 1975. Purine nucleoside phosphorylase from *Escherichia coli* and *Salmonella typhimurium*. *Eur. J. Biochem.,* **51**, 253-265.
11. **Jensen, K.F.,** 1978. Two purine nucleoside phosphorylases in *Bacillus subtilis*. *Biochim. Biophys. Acta,* **525**, 346-356.
12. **Kazmers, I.S., Mitchell, B.S., Dadonna, P.E., Morting, L.L., Townsend, L.B. and W.N. Kelly,** 1981. Inhibition of purine nucleoside phosphorylase by 8-aminoguanosine; Selective toxicity for T lymphoblasts. *Science,* **214**, 1137-1139.
13. **Kalckar, H.M.,** 1947. Differential spectrophotometry of purine compounds by means of specific enzymes. *J. Biol. Chem.,* **167**, 429-443.
14. **Krenitsky, T.A.,** 1976. Uridine phosphorylase from *Escherichia coli*. *Biochim. Biophys. Acta,* **429**, 352-358.
15. **Krenitsky, T.A., G.W. Koszalka and J.V. Tuttle,** 1981. Purine nucleside synthesis, an efficient method employing nucleoside phosphrylases. *Biochemistry,* **20**, 3615-3621.
16. **Lewis, A.S. and B.A. Lowry,** 1979. Human erythrocytic nucleoside phosphorylase; Molecular weight and physical properties. *J. Biol. Chem.,* **254**, 9927-9932.
17. **Lowry, O.H., N.J. Rosebrough, N.J., A.L. Farr and R.J. Randall,** 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* **193**, 265-275.
18. **McRoberts, J.A. and D.W. Martin, Jr.,** 1980. Submolecular characterization of a mutant human purine nucleoside phosphorylase. *J. Biol. Chem.,* **253**, 504-510.
19. **Milman, G.,** 1978. Chinese Hamster purine nucleoside phosphorylase. In: Methods in Enzymology, Vol. 51 (Hoffee, P.A. and M.E. Jones, eds.) pp. 538-543. Academic Press, London.
20. **Moyer, T.P. and A.G. Fischer,** 1976. Purification and characterization of a purine-nucleoside phosphorylase from bovine thyroid. *Arch. Biochem. Biophys.,* **174**, 622-629.
21. **Mura, U., Francesco, S. and P.L. Ipata,** 1978. Utilization of exogenous purine compounds in *Bacillus cereus*. *J. Biol. Chem.,* **253**, 7905-7909.
22. **Osborne, W.R.A.,** 1980. Human red cell purine nucleoside phosphorylase: purification by biospecific affinity chromatography and physical properties. *J. Biol. Chem.,* **255**, 7089-7092.
23. **Robertson, B.C. and P.A. Hoffee,** 1973. Purification and properties of purine nucleoside phosphorylase from *Salmonella typhimurium*. *J. Biol. Chem.,* **248**, 2040-2043.
24. **Schwartz, M.,** 1978. Thymidine phosphorylase from *Escherichia coli*. In: Methods in enzymology, Vol. 51 (Hoffee, P.A. and M.E. Jones and eds.) pp. 442-445. Academic Press, London.
25. **Segel, I.R.,** 1975. In: Enzyme kinetics and behavior and analysis of rapid equilibrium and steady state enzyme systems, pp. 931-934. Wiley, New York.
26. **Stoeckler, J.D. R.P. Argawal, K.C. Argawal and R.E. Park, Jr.,** 1978. Purine nucleoside phosphorylase from human erythrocytes. In: Methods in enzymology, Vol. 51 (Hoffee, P.A. and M.E. Jones, eds.) pp. 530-538. Academic Press, London.
27. **Stoeckler, J.D.,** 1984. Purine nucleoside phosphorylase. In: Developments in Cancer Chemotherapy (Glazer R.I., ed.) pp. 35-60. CRC Press Inc., Boca Raton, F.L.
28. **Stoeckler, J.D., Cambor, C. and R.E. Parks, Jr.,** 1980. Human erythrocytic purine nucleoside phosphorylase: Reaction with sugar modified nucleoside substrates. *Biochemistry,* **19**, 102-107.
29. **Stoeckler, J.D., Cambor, C., Kuhns, V., Chu, S-H. and R.E. Parks, Jr.,** 1982. Inhibitors of purine nucleoside phosphorylase. *Biochem. Phamacol.,* **31**, 163-171.
30. **Umemura, S., Nishino, T., Murakami, K. and K.**

Tsushima, 1982. Trimeric purine nucleoside phosphorylase from chichen liver having a proteolytic nick on each subunit and its kinetic properties. *J. Biol. Chem.*, **257**, 13374-13378.

31. **Van der Weyden, M.B. and L. Bailey,** 1978. A micromethod for determining adenosine deaminase and purine nucleosides phosphorylase activity in cells from peripheral blood. *Clin. Chim. Acta.* **82**. 179-184.
32. **Vogels, G.D. and C. Van der Drift,** 1976. Degradation of purines and pyrimidines by microorganisms. *Bacteriol. Rev.*, **40**, 191-202.
33. **Wiginton, D.A., Coleman, M.S. and J.J. Hutton,** 1980. Characterization of purine nucleoside phosphorylase from human granulocytes and its metabolism of deoxyribonucleosides. *J. Biol. Chem.*, **255**, 6663-6669.
34. **Zannis, V.I., Doyle, D. and Martin, D.W., Jr.,** 1978. Purification and characterization of human erythrocyte purine nucleoside phosphorylase and its subunits. *J. Biol. Chem.*, **253**, 504-510.

(Received May 7, 1991)
(Accepted June 3, 1991)