Steady-State Kinetics of the Schistosomal Hypoxanthine-Guanine Phosphoribosyltransferase†

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ABSTRACT: Schistosomiasis is a trematode infection of some 200 million people. The hypoxanthine–guanine phosphoribosyltransferase (HGPRTase) of the major etiologic agent, *Schistosoma mansoni*, has been proposed as a potential target for antischistosomal chemotherapy [Dovey, H. F., McKerrow, J. H., & Wang, C. C. (1984) *Mol. Biochem. Parasitol. 11*, 157–167]. The steady-state kinetic mechanism for the schistosomal HGPRTase has been determined by including both hypoxanthine and guanine in the forward and reverse reactions under identical conditions. Double-reciprocal plots of initial velocity versus the concentration of one substrate, at a series of fixed concentrations of the other, give groups of intersecting straight lines indicating a sequential mechanism for the schistosomal HGPRTase-catalyzed reactions. In product inhibition studies, the results show that magnesium pyrophosphate (MgPPi) is a noncompetitive inhibitor with respect to dimagnesium phosphoribose pyrophosphate (Mg_2PRPP), hypoxanthine, and guanine. Also, magnesium inosine monophosphate (MgIMP) and magnesium guanosine monophosphate (MgGMP) are noncompetitive inhibitors with respect to hypoxanthine or guanine, respectively, but are competitive inhibitors to Mg_2PRPP. Furthermore, Mg_2PRPP is a competitive inhibitor with respect to MgIMP and MgGMP but is a noncompetitive inhibitor to MgPPi. The minimum kinetic model which fits the experimental data is an ordered bi-bi mechanism, where the substrates bind to the enzyme in a defined order (first Mg_2PRPP followed by the purine bases), while products are released in sequence (first MgPPi followed by MgIMP or MgGMP). The ordered release of products of the schistosomal enzyme apparently is different from that reported for the human HGPRTase in which the release of products is in rapid equilibrium random fashion [Giacomello, A., & Salerno, C. (1978) *J. Biol. Chem. 253*, 6038–6044]. These results suggest that the design of a highly specific inhibitor of the schistosomal HGPRTase, which binds exclusively to the enzyme–purine nucleotide binary complex, may be possible.

Hypoxanthine–guanine phosphoribosyltransferase (HGPRTase;[1] EC 2.4.2.8) catalyzes the removal of the pyrophosphate moiety from PRPP and the attachment of a purine base (hypoxanthine or guanine) in an apparent SN2 reaction to a form purine nucleotide (IMP or GMP) and pyrophosphate (PPi). HGPRTase is one member of a class of approximately 10 phosphoribosyltransferases (PRTases) that catalyze similar reactions involving PRPP and a nitrogenous base (Musick, 1981). The mechanisms for the majority of the PRTase-catalyzed reactions remain largely unknown. A ping-pong kinetic mechanism has been proposed for the yeast OPRTase (Victor et al., 1979) and the yeast UPRTase (Natalini et al., 1979). In some cases, an enzyme-bound 5-phosphoribosyl intermediate has been identified (Victor et al., 1979; Ali & Sloan, 1982), supporting the ping-pong model. However, many PRTases appear to follow sequential kinetic mechanisms (Musick, 1981; Giacomello & Salerno, 1978; Ali & Sloan, 1982; Bhatia et al., 1990), in which both ordered and random binding of substrates or release of products have been demonstrated. Clearly, despite the similarities in the amino acid sequences for these enzymes, there is no single kinetic mechanism that can be used to define the PRTase-catalyzed reactions.

Schistosoma mansoni is a human parasite causing schistosomiasis, which is one of the most prevalent infectious diseases in the world. The schistosomal HGPRTase is apparently crucial for the survival of this pathogen because of the incapability of de novo synthesis of purine nucleotides and therefore has been proposed as a potential target for antischistosomal chemotherapy (Senft & Crabtree, 1983; Dovey et al., 1984). Since defects in the human HGPRTase are known to be responsible for gout and Lesch–Nyhan syndrome (Kelley et al., 1969), it is imperative that effective drugs not severely inhibit the human enzyme. Therefore, extensive kinetic analyses of both HGPRTases are necessary to explore any potential difference in the active sites and catalytic mechanisms in order to design specific inhibitors that might be useful as antischistosomal drugs. We have expressed cDNA encoding the enzyme in *Escherichia coli*, and the recombinant enzyme thus generated was shown to be physically and kinetically identical to the native enzyme (Yuan et al., 1990; Craig et al., 1991). This has enabled us to purify large quantities of the schistosomal HGPRTase for more detailed steady-state kinetic studies. The availability of large quantities of the purified schistosomal HGPRTase enabled us to include hypoxanthine

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[1] Abbreviations: HGPRTase, hypoxanthine–guanine phosphoribosyltransferase; HPRTase, hypoxanthine phosphoribosyltransferase; GPRTase, guanine phosphoribosyltransferase; PRPP, 5-phosphoribosyl pyrophosphate; IMP, inosine monophosphate; GMP, guanosine monophosphate; PPi, pyrophosphate; OPRTase, orotate phosphoribosyltransferase; UPRTase, uracil phosphoribosyltransferase; HPLC, high-pressure liquid chromatography; U, enzyme unit, 1 U is defined as the conversion of 1.0 μmol of substrate per min.

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and guanine in both forward and reverse reactions under identical experimental conditions. Our results favor an ordered bi-bi mechanism where PRPP binds to the enzyme first and the product IMP or GMP is released last, which is apparently different from that reported for the human HGPRTase (Giacomello & Salerno, 1978).

Materials and Methods

Hypoxanthine, guanine, IMP, GMP, and pyrophosphate were purchased from Sigma in the highest purities available. Xanthine oxidase (20 U/mL) was from Boehringer Mannheim, and guanase (1.3 U/mL) was from Sigma. Both commercial enzymes were dialyzed against buffer containing 100 mM Tris-HCl, pH 7.4, and 12 mM $MgCl_2$. The dimagnesium salt of PRPP (Mg_2PRPP) was from ICN Biochemicals, Inc., and was determined to be 1.5 μmol/mg using the assay described by Kornberg et al. (1955). Paper chromatography, performed according to Wood (1968), showed no significant impurity in the commercial PRPP.

Enzyme Purification. The recombinant schistosomal HGPRTase was purified from low-phosphate-induced *E. coli* strain SØ606 (*Δgpt-pro-lac, thi, hpt*) transformed with the pBSprt expression plasmid (Craig et al., 1991). Cells were grown in low-phosphate medium (Yuan et al., 1990) at 37 °C for 16 h and then pelleted and suspended in 100 mM Tris-HCl, pH 7.4, and 12 mM $MgCl_2$, and lysed by sonication. Nucleic acids were precipitated by protamine sulfate (Sigma) at a ratio of 1:20 (w/w, protamine sulfate/total proteins). After centrifugation at 5000*g* for 5 min, the supernatant was heated to 80 °C for 2 min. Using procedures described before (Yuan et al., 1990), the denatured proteins were removed by centrifugation, and the supernatant was loaded onto a HPLC Mono Q column (Pharmacia). The enzyme-activity-containing fractions were further fractionated on a Mono P column (Pharmacia). The purified HGPRTase was determined to be more than 99% pure by silver-stained SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

Enzyme Assay. Kinetic experiments were performed using a Beckman DU-7 spectrophotometer equipped with a kinetics accessory. The formations of IMP and GMP were followed spectrophotometrically at 245 and 257.5 nm, respectively (Hill, 1970). All measurements were carried out in 100 mM Tris-HCl, pH 7.4, and 12 mM $MgCl_2$ at 37 °C. Under these conditions, the extinction coefficients for IMP and GMP were 3020 ± 40 and 5340 ± 50 M^{-1} cm^{-1}, respectively. The coefficients of variation were 4.2% for the assays at 245 nm and 3.9% for the assays at 257.5 nm when 10 measurements for each assay were performed within 3 h. The final volume of assay mixture, containing various amounts of substrates, was 1.0 mL.

IMP pyrophosphorolysis was monitored spectrophotometrically as described previously (Giacomello & Salerno, 1978). The production of hypoxanthine was determined indirectly by the continuous spectrophotometric assay of uric acid formation in the presence of xanthine oxidase. The assay mixture contained 100 mM Tris-HCl, pH 7.4, 12 mM $MgCl_2$, and 0.02 U/mL xanthine oxidase. The reaction was initiated by addition of the purified HGPRTase, and was monitored at 293 nm at 37 °C. A molar extinction coefficient of 12 000 M^{-1} cm^{-1} was assumed for uric acid at 293 nm (Kalckar, 1947). GMP pyrophosphorolysis was determined by the continuous spectrophotometric assay of uric acid formation in the presence of both guanase (0.01 U/mL) and xanthine oxidase (0.02 U/mL). Other conditions were as described for the IMP pyrophosphorolysis assay. All experiments were performed in quadruplicate.

In determining the initial velocities for the forward reactions, when either hypoxanthine or guanine was the variable substrate (1–50 μM), six different fixed concentrations of Mg_2PRPP (10–100 μM) were used; and when Mg_2PRPP was the variable substrate (10–150 μM), seven different fixed concentrations of hypoxanthine or guanine (2–50 μM) were used. In the reverse reactions, when either MgIMP or MgGMP was the variable substrate (10–200 μM), six different fixed concentrations of MgPPi (20–250 μM) were used; and when MgPPi was the variable substrate (10–200 μM), six different fixed concentrations of MgIMP or MgGMP (15–500 μM) were used.

The dimagnesium salt of PRPP (Mg_2PRPP) and monomagnesium complexes of IMP, GMP, and PPi (MgIMP, MgGMP, and MgPPi, respectively) have been demonstrated to be the true substrates of HGPRTase (Salerno & Giacomello, 1981). Therefore, the concentrations of these complexes used in data analysis were calculated as described previously, assuming that no Mg^{2+} complexes with hypoxanthine or guanine are formed (Giacomello & Salerno, 1978; Salerno & Giacomello, 1981).

Data Analysis. Initial rate data were fitted to the following equations (Cleland, 1963) using kinetics software from BioMetallics, Inc. (K-CAT) and Hewlett-Packard (HP89512A). For competitive inhibition

$$v = V_{max}S/[K_{m,app}(1 + I/K_{is}) + S]$$

For noncompetitive inhibition

$$v = V_{max}S/[K_{m,app}(1 + I/K_{is}) + S(1 + I/K_{ii})]$$

For uncompetitive inhibition

$$v = V_{max}S/[K_{m,app} + S(1 + I/K_{ii})]$$

The best fits were determined by weighting constant relative error [$w \approx 1/[(\text{relative error})V_o]^2$] and constant absolute error [$w \approx 1/(\text{absolute error})^2$]. The nomenclature is that of Cleland (1963): v, velocity; V_{max}, maximum velocity; S, substrate concentration; $K_{m,app}$, apparent Michaelis constant; K_{is} and K_{ii}, slope and intercept inhibition constants, respectively; I, inhibitor concentration. The slopes and intercepts of the replots were calculated using weighted linear regression. Weights were calculated from the standard errors of $K_{m,app}$, $1/V_{max}$, or $1/K_{m,app}$, and the standard errors are indicated by error bars in the replots.

Results

Using the purification procedures described here, we purified more than 15 mg of the schistosomal HGPRTase from a 1-L culture of induced bacteria containing the pBSprt expression plasmid. The purified enzyme is stable at −20 °C for more than two months in the presence of 50% glycerol.

We have studied the steady-state kinetics of synthesis and pyrophosphorolysis of IMP and GMP and the product inhibitions of these reactions to identify the order of substrate binding and product release. The initial rates of these reactions were proportional to the enzyme concentration. Free Mg^{2+} ion concentrations were always held constant at 12 mM, which was at least 30 times higher than the concentrations of all substrates tested. Under these conditions, the concentration of Mg-complexed substrate was determined by the association constant (Giacomello & Salerno, 1978; Switzer, 1971) and was proportional to the total amount of substrate present in solution. Guanase (EC 3.5.4.3) deaminates guanine to form xanthine, and the guanase and xanthine oxidase coupled assay system for the GMP phosphorolysis enabled us to monitor the

Table I: Kinetic Constants of the Schistosomal HGPRTase[a]

	K_m (μM)	K_s (μM)
(A) GPRTase		
Mg_2PRPP	18.2 ± 1.3	44.2 ± 15.1
guanine	3.0 ± 0.2	60.6 ± 13.1
MgPPi	22.7 ± 0.5	134.5 ± 21.5
MgGMP	8.4 ± 0.8	63.5 ± 12.5
(B) HPRTase		
Mg_2PRPP	9.3 ± 1.1	69.9 ± 23.1
hypoxanthine	5.4 ± 0.2	137.5 ± 13.8
MgPPi	25.6 ± 1.3	161.1 ± 31.4
MgIMP	5.7 ± 0.4	86.9 ± 15.6

[a] Experimental conditions: 100 mM Tris-HCl, pH 7.4, 12 mM $MgCl_2$, at 37 °C; other conditions are as described under Materials and Methods.

reaction spectrophotometrically. The schistosomal HGPRTase has a broad pH optimum of 6.9–7.5 (Yuan et al., unpublished data). The assay conditions for the present studies of the schistosomal HGPRTase (100 mM Tris-HCl, pH 7.4, 12 mM $MgCl_2$, at 37 °C) were identical to those used for the human HGPRTase (Giacomello & Salerno, 1978), allowing direct comparison of the kinetic properties between the two enzymes.

Steady-State Kinetics. In both forward and reverse reactions of the schistosomal HGPRTase, double-reciprocal plots of initial velocity versus the concentration of one substrate, at a series of fixed concentrations of the other, give groups of intersecting straight lines (Figure 1). The data obtained for the forward reactions show that the lines are intersecting below the 1/[S] axis, fitting with either a ping-pong or a sequential model in which $K_{m,app}$ values are higher than the dissociation constants (K_s). However, the reverse reactions show clearly intersecting patterns above the 1/[S] axis (data not shown), strongly indicating a sequential mechanism. No substrate inhibition was observed in these studies.

The values of the Michaelis constant (K_m) and dissociation constant (K_s) for the four substrates and products were calculated from initial velocity and product inhibition experiments according to the methods of Ainsworth (1977) and are given in Table I. Consistent with our previous report (Yuan et al., 1990), guanine is a better substrate than hypoxanthine for the schistosomal HGPRTase, since the enzyme, under identical assay conditions, has a lower K_m (3.0 ± 0.2 μM) and a higher k_{cat} (5.7 ± 0.3 s^{-1}) for guanine than hypoxanthine (K_m = 5.4 ± 0.2 μM; k_{cat} = 2.8 ± 0.1 s^{-1}). The V_{max} values are 6.5 and 8.7 μmol/(min·mg) of protein for hypoxanthine and guanine,

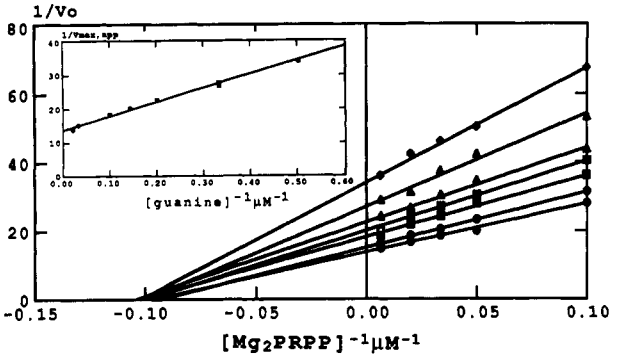


FIGURE 1: Initial velocity pattern for the forward reaction with Mg_2PRPP as the variable substrate (10–150 μM) at different fixed concentrations of guanine (2–50 μM). All other conditions were as described under Materials and Methods. (Inset) Replot of $1/V_{max,app}$ with respect to the reciprocal micromolar concentration of guanine.

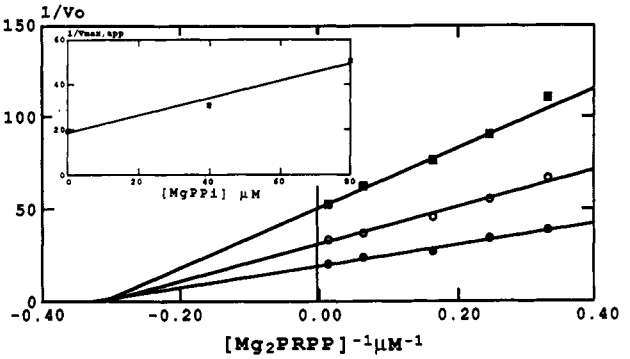


FIGURE 2: Product inhibition of the forward reaction by MgPPi with Mg_2PRPP as the variable substrate. The concentration of guanine was held constant at 70 μM. The concentrations of MgPPi were (●) none; (○) 40 μM; (■) 80 μM. All other conditions were as described under Materials and Methods. (Inset) Replot of $1/V_{max,app}$ with respect to the micromolar concentrations of MgPPi.

respectively. The ratios between the maximal initial velocities of the forward and reverse reactions ($V_{forward}/V_{reverse}$) were 42 ± 2 for hypoxanthine and 38 ± 2 for guanine.

Product Inhibition. To determine the binding order for substrates of the schistosomal HGPRTase-catalyzed reaction, we performed product inhibition experiments with the products of both the forward and reverse reactions (Table II). All inhibition patterns are linear. In the forward reaction for GMP synthesis, MgPPi is noncompetitive versus both Mg_2PRPP (K_{ii}

Table II: Product Inhibition of GPRTase and HPRTase Activities in both Forward and Reverse Reactions[a]

inhibitor	varied substrates	fixed substrate (μM)	pattern type	K_{ii} (μM)	K_{is} (μM)
(A) GPRTase					
MgPPi	Mg_2PRPP	guanine (70)	NC	49 ± 7	46 ± 6
MgPPi	guanine	Mg_2PRPP (150)	NC	82 ± 9	112 ± 11
MgGMP	Mg_2PRPP	guanine (70)	C	–	55 ± 2
MgGMP	guanine	Mg_2PRPP (150)	NC	87 ± 3	70 ± 2
Mg_2PRPP	MgPPi	MgGMP (250)	NC	63 ± 2	248 ± 40
Mg_2PRPP	MgGMP	MgPPi (150)	C	–	31 ± 1
guanine	MgPPi	MgGMP	–	–	–
guanine	MgGMP	MgPPi	–	–	–
(B) HPRTase					
MgPPi	Mg_2PRPP	hypoxanthine (80)	NC	120 ± 17	490 ± 130
MgPPi	hypoxanthine	Mg_2PRPP (150)	NC	84 ± 13	114 ± 1
MgIMP	Mg_2PRPP	hypoxanthine (80)	C	–	46 ± 6
MgIMP	hypoxanthine	Mg_2PRPP (150)	NC	68 ± 2	519 ± 8
Mg_2PRPP	MgPPi	MgIMP (250)	NC	59 ± 2	126 ● 34
Mg_2PRPP	MgIMP	MgPPi (150)	C	–	35 ± 3
hypoxanthine	MgPPi	MgIMP	–	–	–
hypoxanthine	MgIMP	MgPPi	–	–	–

[a] Experimental conditions are as described under Materials and Methods. NC, noncompetitive inhibition; C, competitive inhibition; K_{ii} and K_{is}, intercept and slope inhibition constants, respectively; –, not determined.

= 49 ± 7 μM, K_{is} = 46 ± 6 μM; Figure 2) and guanine (K_{ii} = 82 ± 9 μM, K_{is} = 112 ± 11 μM), while MgGMP is competitive versus Mg_2PRPP (K_{is} = 55 ± 2 μM) but noncompetitive versus guanine (K_{ii} = 87 ± 3 μM, K_{is} = 70 ± 2 μM). In agreement with these results, in the forward reaction for IMP synthesis, MgPPi is also noncompetitive versus Mg_2PRPP and hypoxanthine, whereas MgIMP is competitive versus Mg_2PRPP but noncompetitive versus hypoxanthine. In GMP pyrophosphorolysis, Mg_2PRPP is noncompetitive with respect to MgPPi (K_{ii} = 63 ± 2 μM, K_{is} = 248 ± 40 μM) but competitive to MgGMP (K_{is} = 31 ± 1 μM). Consistent with this result, in IMP pyrophosphorolysis Mg_2PRPP is noncompetitive versus MgPPi but competitive versus MgIMP. Studies of potential inhibitions of the reverse reactions by hypoxanthine and guanine were prevented by the presence of activities of xanthine oxidase and guanase, respectively. Also, analyses of product inhibitions in saturating concentrations of the substrates were hampered by the insolubilities of the bases and the high UV absorbance of these compounds at the wavelengths employed to monitor the reactions. Nevertheless, our results, taken together, are consistent with an ordered sequential mechanism including an E–PRPP–base and an E–PPi–nucleotide intermediate.

Discussion

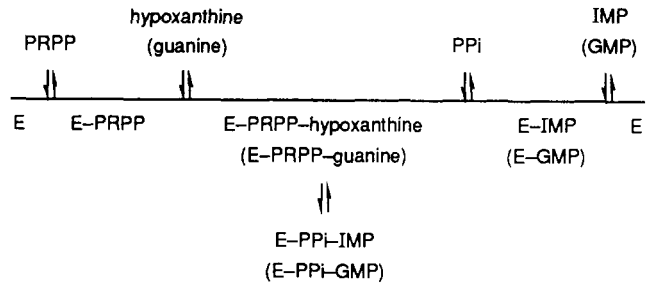
Until very recently, most kinetic studies of PRTases have been hindered by insufficient quantities of purified enzyme. Our success in constructing a novel expression system that produces more than 50 mg of soluble enzymatically active schistosomal HGPRTase per liter of culture of transformed *E. coli* (Craig et al., 1991) has enabled us to overcome this problem.

Double-reciprocal plots of initial velocities versus the concentrations of one substrate, at a series of fixed concentrations of the other, gave groups of intersecting straight lines, indicating a sequential mechanism for the schistosomal HGPRTase-catalyzed reaction. Under the same assay conditions, Lineweaver–Burk plots generated similar intersecting straight lines for the human HGPRTase (Giacomello & Salerno, 1978). In order to identify whether the sequential mechanism is ordered or random, we performed product inhibition experiments. The results show that MgPPi is a noncompetitive inhibitor with respect to Mg_2PRPP, hypoxanthine, and guanine. Also, MgIMP and MgGMP are noncompetitive inhibitors with respect to hypoxanthine or guanine, respectively, but are competitive inhibitors to Mg_2PRPP. Furthermore, Mg_2PRPP is a competitive inhibitor with respect to MgIMP and MgGMP but is a noncompetitive inhibitor to MgPPi. The minimum kinetic model which fits the experimental data is an ordered bi-bi mechanism (Cleland, 1979), where the substrates bind to the enzyme in a defined order (first Mg_2PRPP followed by the purine bases), while products are released in sequence (first MgPPi followed by MgIMP or MgGMP) (Scheme I).

As shown in Scheme I, only PRPP and the purine nucleotides can bind to the free enzyme (E). In contrast, the purine bases only bind to the E–PRPP complex. This is consistent with the results from studies of the yeast HGPRTase where the E–PRPP complex was identified while enzymic complexes with either hypoxanthine or guanine were not detected in flow dialysis experiments (Ali & Sloan, 1982). Furthermore, our results argue against PPi binding to either E or E–PRPP.

The literature has been inconsistent on the mechanism of the human HGPRTase-catalyzed reaction. Studies of the human HGPRTase (Krenitsky & Papaioannou, 1969) suggest that an enzyme-bound 5-phosophoribosyl intermediate may

Scheme I: Ordered Bi-Bi Mechanism for the Schistosomal HGPRTase



exist in the reaction. This type of intermediate has also been demonstrated in the yeast OPRTase (Victor et al., 1979) and the OPRTase of *Salmonella typhimurium* (Bhatia et al., 1989), suggesting a ping-pong kinetic mechanism with PPi dissociating from the active site prior to the binding of the base. However, more careful studies of the OPRTase of *S. typhimurium* by Bhatia et al. (1990) showed that the PRPP–PPi exchange was a result of contamination, and therefore the authors suggest that previous studies of other PRTases may have been subject to similar artifacts. A detailed study on the formation and phosphorolysis of IMP catalyzed by the human HGPRTase (Giacomello & Salerno, 1978) supports a sequential mechanism. For their study, the initial velocity data from both forward and reverse reactions, at essentially constant free Mg^{2+} ion concentrations, produced a series of lines that intersected to the left of the $1/v$ axis. Also, for product inhibition studies, both MgIMP and MgPPi were competitive inhibitors of Mg_2PRPP but were noncompetitive inhibitors of hypoxanthine, while Mg_2PRPP was a competitive inhibitor with respect to MgIMP and MgPPi. These data strongly supported an ordered bi rapid equilibrium random bi mchanism, where Mg_2PRPP binds to the enzyme first followed by hypoxanthine, and the products (MgIMP and MgPPi) are released in random fashion.

In the present study of the schistosomal HGPRTase, all data are consistent with an ordered bi-bi mechanism. The results of our product inhibition studies either using purine nucleotides (IMP or GMP) to compete with purine bases (hypoxanthine or guanine) and PRPP or using PPi to compete with purine bases are in agreement with those obtained from the human enzyme (see above). However, in contrast to the human enzyme, our data indicate that PPi and the purine nucleotides do not bind to the same enzyme form, suggesting that the release of products is sequential with IMP (or GMP) being released last.

Giacomello and Salerno (1978) argued that the human HGPRTase could not proceed via an ordered bi-bi mechanism. They arrived at this conclusion because in an ordered bi-bi reaction the abscissa at the point of intersection of the straight lines of the double-reciprocal plot of initial velocity against PRPP concentration, at a series of purine base concentrations, is given as

$$A_1 = -\frac{1}{K_{s,PRPP}}$$

while at constant concentration of purine base and different fixed concentrations of PPi, the abscissa is given as

$$A_2 = -\frac{1}{K_{s,PRPP}}\left(1 + \frac{K_{m,PPi}K_{i,\text{purine nucleotide}}[\text{base}]}{K_{m,base}K_{m,\text{purine nucleotide}}K_{i,PPi}}\right)$$

Because the value in parentheses is always greater than 1, $A_1 > A_2$. This condition was not fulfilled in the data reported

for the human enzyme (Henderson et al., 1968; Giacomello & Salerno, 1978), and thus the human HGPRTase may not be accurately defined by an ordered bi-bi mechanism. In contrast, as shown in Figures 1 and 2 of this report, A_1 is greater than A_2 for the schistosomal HGPRTase.

Our results indicate that the schistosomal HGPRTase may preceed via a different kinetic mechanism, under the same assay conditions, than does the human counterpart. This difference between the two enzymes may facilitate the design of specific inhibitors for the schistosomal HGPRTase that might have potential as antischistosomal drugs. Since the ordered binding of substrates and release of products demands the existence of discrete binary complexes of the schistosomal HGPRTase with PRPP or purine nucleotides, the design of inhibitors which selectively bind to either of these forms should be possible. Inhibitors which bind exclusively to the E–purine nucleotide forms should have higher specificity for the schistosomal HGPRTase than for the human enzyme. Also, understanding details of the kinetic mechanism should help us to predict the inhibition patterns of different potential inhibitors. In order to verify the kinetic mechanism for the human HGPRTase, we are currently expressing the human HGPRTase in *E. coli* using the same expression system used for the schistosomal enzyme.

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