Artemia purine phosphoribosyltransferases

Purification and characterization

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Adenine phosphoribosyltransferase (APRTase) and hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) have been purified from *Artemia* cysts and nauplii to apparent homogeneity, as determined by SDS-PAGE. The purification includes affinity chromatography on AMP-Sepharose, which binds both enzymes, and they are eluted at different 5-phospho- α -D-ribose diphosphate (*PP*-Rib-*P*) concentrations. The purified enzymes from *Artemia* cysts were similar to nauplii enzymes with respect to M_r in denaturing gel electrophoresis and gel filtration, pH and cation dependence and kinetic constants for substrates and inhibitors. By Sephadex G-100 filtration, the native M_r of the adenine and hypoxanthine-guanine enzymes was estimated to be M_r 28 000 and 66 000, respectively. Analysis by SDS-PAGE revealed that the APRTase was a dimer of M_r 15 000 sub-units and the HGPRTase, a tetramer of four identical M_r 19 000 sub-units. The pH profile of the HGPRTase shows two apparent buffer-independent pH optima, at 7.0 and 9.5, while the APRTase has just one, at about pH 8–9. The purine phosphoribosyltransferase activity with adenine was highest, about tenfold the HGPRTase activity with hypoxanthine and fivefold that with guanine. Both enzymes exhibited similar requirements for divalent cations, either Mg^{2+} , Mn^{2+} or Zn^{2+} , while Ca^{2+} is highly inhibitory. The K_m values of APRTase for adenine and *PP*-Rib-*P* are 2 and 30 μM , respectively, and the K_m values of HGPRTase for hypoxanthine, guanine and *PP*-Rib-*P* are < 1, < 1 and 15 μM , respectively. Plots of the reciprocal enzyme activities versus reciprocal concentrations of one substrate at several fixed levels of the second one yield a pattern of inhibition by guanine and hypoxanthine. Product-inhibition studies indicated that AMP is a competitive inhibitor with respect to *PP*-Rib-*P* in the APRTase reaction, while the HGPRTase shows a mixed inhibition by GMP.

INTRODUCTION

In the purine salvage synthesis pathways, the phosphoribosyltransferase enzymes play an important role both in the incorporation or uptake of the adenine, hypoxanthine and guanine bases [1,2] and in the control of these functions through activator or inhibitor mechanism [3,4].

The biosynthesis, metabolism and interconversion of purine nucleotides in *Artemia* have been the subject of study and controversy, given the apparent lack of *de novo* synthesis [5,6] and the accumulation in cysts of large quantities of guanine derivatives [7] mainly P^1, P^4 -diguanosine tetraphosphate (Gp^4G), which are metabolised by interconversion throughout the development of *Artemia* [8–10].

Previous work from this laboratory has shown the *in vivo* capacity of *Artemia* nauplii to synthesise purines through the *de novo* pathway [11] and also the incorporation of the adenine, hypoxanthine and guanine bases into the corresponding nucleotides through the salvage route [12–14]. Thus, our studies have been directed towards determining the capacity of this system for purine synthesis and evaluating the relative importance of the salvage pathways, which correspond to approx. 80% of total nucleotide synthesis.

Here the objective is the study of the physico-chemistry, kinetics and regulatory properties of the *Artemia* phosphoribosyltransferases, to better understand the function of the salvage pathways at the molecular level of the enzymes and their possible changes during development.

MATERIALS AND METHODS

Materials

[8- ^{14}C]Hypoxanthine (50 mCi/mmol) and [8- ^{14}C]adenine were obtained from Amersham and [8- ^{14}C]guanine from New England Nuclear. 5-Phospho- α -D-ribose diphosphate (*PP*-Rib-*P*) (sodium salt), nucleotides, bases and AMP-Sepharose were from Sigma Chemical Co. Molecular-mass standards for gel filtration and SDS-PAGE were purchased from Pharmacia and Sigma Chemical Co. respectively. All other materials were of analytical grade and of the highest commercially available purity.

Enzyme source

Dried *Artemia* cysts were supplied by the San Francisco Bay Brand Co., Menlo Park, CA, U.S.A. Treatment of the cysts and growth conditions, as well as isolation and development of newborn nauplii, were performed as described by Llorente & Cardaba [15].

Enzyme purification

Wet cysts (10 g) or 40-h-old nauplii, obtained as described above, were homogenized with 2 vol. of 50 mM-Tris/HCl (pH 7.5) buffer containing 100 mM-KCl, 1 mM-MgCl₂ and 0.2 mg of soybean trypsin inhibitor (type I.S.). The homogenate was centrifuged at 100 000 *g* for 60 min. the supernatant was precipitated with a saturated (NH₄)₂SO₄ solution.

The 40–80%-satd. (NH₄)₂SO₄ pellet, which contained most of the enzyme activity, was collected and resuspended in a minimum

Abbreviations used: Gp^4G , 5',5'''- P^1, P^4 -diguanosine tetraphosphate; Gp^3G , 5',5'''- P^1, P^3 -diguanosine triphosphate; APRTase, adenine phosphoribosyltransferase (EC 2.4.2.7); HGPRTase, hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8); HPRTase, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8); GPRTase, guanine phosphoribosyltransferase (EC 2.4.2.8), *PP*-Rib-*P*, 5-phospho- α -D-ribose diphosphate.

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Table 1. Purification of APRTase and HGPRTase from *Artemia* cysts and nauplii

Details of the purification are given in the Materials and methods section. Enzyme activities were determined under standard assay conditions and expressed in terms of units μmol of mononucleotides (AMP, GMP and IMP formed)/minute at 37 °C

Fraction	APRTase activity			HGPRTase activity			
	(munits)	(munits/mg)	Yield (%)	(munits*)	(munits/mg)	Yield (%)	Guanine/hypoxanthine†
100000 g							
Cysts	275	5	100	11	0.2	100	2.0
Nauplii	263	4	100	15	0.3	100	2.1
(NH ₄) ₂ SO ₄							
Cysts	276	32	100	14	1.7	100	2.3
Nauplii	262	21	100	12	1	80	2.0
Sepharose-AMP							
Cysts	54	13 300	20	3	500	27	2.0
Nauplii	80	14 200	30	5	650	35	2.0

* Phosphoribosyltransferase activity with hypoxanthine.

† Ratio of the activity with guanine to the activity with hypoxanthine.

Fig. 1. Elution profile of APRTase and HGPRTase from *Artemia* cysts and/or nauplii by AMP-Sepharose affinity chromatography

Phosphoribosyltransferase activities with adenine, hypoxanthine and guanine were measured in the fractions collected as described under Materials and methods. The amount of protein used for the HGPRTase activity assay was about tenfold that of the APRTase. Column buffers and flow rates are described in the text. The arrows indicate the points at which KCl (0.2 mM) and PP-Rib-P (5.0 mM) were added to elute the enzymes. ○, APRTase; ●, HPRase; △, GPRase.

volume of 10 mM-Tris/HCl, pH 7.0, containing 5 mM-MgCl₂, 1 mM-dithioerythritol and 20 % glycerol (buffer A). This solution was desalted on a Sephadex G-25 column (1 cm × 20 cm) which had previously been equilibrated with 10 vol. of buffer A. The desalted enzyme solution was applied to a AMP-Sepharose (N⁶-aminohexyl-5'-AMP-Sepharose 4B) column (0.8 cm × 6 cm), previously equilibrated with buffer A, and allowed to enter the column for 60 min. The column was then washed at a flow rate of 4 ml/h with buffer A and with the same buffer containing 0.1 M-KCl to remove unbound proteins. The APRTase and HGPRTase activities were recovered by the

Fig. 2. SDS-PAGE of purified APRTase and HGPRTase from *Artemia* cysts and nauplii (see Materials and methods)

Lanes 1–4 show peak-activity fractions from the AMP-Sepharose column: 10 μg APRTase from cysts (lane 1); 10 μg APRTase from nauplii (lane 2); 2 μg HGPRTase from cysts (lane 3); 2 μg HGPRTase from nauplii (lane 4). Proteins were detected by a silver staining procedure. The electrophoresis mobilities and M_r of standard proteins are indicated. Proteins used as standards were, in descending M_r , bovine albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, α -lactalbumin and horse heart cytochrome c.

addition of 0.2 mM and 5 mM-PP-Rib-P, respectively. Fractions containing activity were pooled and assayed for total enzyme activity. For the total protein assay, the suspensions were dialysed against 1 l of buffer A (without glycerol) overnight and concentrated by lyophilization.

Fig. 3. M_r determination by gel filtration

APRTase and HGPRTase from cysts and/or nauplii were passed through a Sephadex G-150 column and 1.0 ml fractions were collected and assayed for activities as described in the Materials and methods section. The column (0.7 cm $\times$ 2.6 cm), with a flow rate of 4.0 ml/h, was equilibrated in 10 mM-Tris/HCl, pH 7.0, 5 mM-MgCl₂, 1 mM-dithioerythritol and 20% glycerol, and calibrated with trypsinogen (M_r 24000), ovalbumin (M_r 45000), BSA (M_r 66000) and phosphatase (M_r 100000). The void volume was determined using Dextran Blue (M_r 2×10^6). Standards and samples were applied in 1.0 ml of elution buffer. The arrows show the APRTase and HGPRTase positions on the standard curve. $\blacktriangle$, Cysts; $\square$, nauplii.

Enzyme assays

APRTase and HGPRTase activities were assayed routinely by determining the nucleotide formation from the radiolabelled bases by a modification of the method described by Rotllan & Miras [16]. All assays were performed at 37 °C. The standard reaction mixture (100 μ l) contained 50 mM-Tris-HCl, pH 8, 1 mM-MgCl₂, 1 mM-PP-Rib-P and 25 μ M-[8-¹⁴C]adenine (0.14 mCi/mmol) for APRTase assays, and 4 μ M of either [¹⁴C]hypoxanthine or [¹⁴C]guanine (0.14 and 0.2 mCi/mmol, respectively) for HGPRTase assays. The reaction was initiated by the addition of the enzyme and stopped by adding 10 μ l of 0.2 M-EDTA. The reaction mixture (50 μ l) was spotted on to DE 81 paper, dried, washed and counted for radioactivity as described [16]. Unless otherwise stated, all assays were carried out at pH 7.5 and incubations were 10–15 min.

PAGE

SDS-PAGE was performed according to Laemmli [17]. Protein samples were treated for 10 min at 100 °C in 62.5 mM-Tris (pH 6.8)/10% (w/v) SDS/25% (v/v) 2-mercaptoethanol/50% (v/v) glycerol/0.005% Bromophenol Blue and then loaded on to 1% SDS/5–20% (w/v)-acrylamide gradient slab gels which were run at a constant current of 8 mA or on to 10%-acrylamide gels. Molecular-mass standards were run in parallel. Following electrophoresis, proteins were silver-stained as described [18].

Protein determinations

Determination of protein concentration of purified enzymes were performed using the Bradford method [19], with BSA as standard. Protein concentrations of the 100000 *g* supernatants and (NH₄)₂SO₄ fractions were determined as described by Lowry *et al.* [20].

Fig. 4. Effect of pH on the activity of APRTase and HGPRTase from *Artemia* cysts (a) and nauplii (b)

The complete assay mixture was as described in the Materials and methods section, except that the buffer used was 100 mM-imidazole/HCl (open symbols) or 50 mM-Tris/HCl (closed symbols). ($\bullet$, $\circ$), APRTase; ($\blacktriangle$, $\triangle$), HGPRTase.

RESULTS

Purification of APRTase and HGPRTase

Table 1 summarises the results of a typical purification of APRTase and HGPRTase activities from *Artemia* cysts and nauplii. The specific activities of the 100000 *g* supernatant were approx. 5 and 0.2 nmol/min per mg for adenine and hypoxanthine, respectively, from cysts, and 4.0 and 0.3 nmol/min per mg from nauplii. The proteins were precipitated at 40–80% saturation with (NH₄)₂SO₄ and about a fivefold enrichment of specific activity was obtained with practically total recovery of the activity.

Following gel filtration on Sephadex G-25, the adenine and hypoxanthine-guanine enzyme activities were separated by affinity chromatography on a Sepharose-AMP column, which binds both phosphoribosyltransferases; the enzyme activities

Table 2. Summary of kinetic constants of APRTase and HGPRTase activities from *Artemia* cysts and nauplii

In each case saturating concentrations of the rest of substrates were used. The conditions of the assay are described in the Materials and methods section. Values are the mean of four determinations, which varied < 10 %. A, APRTase activity; H and G, HGPRTase activity in the presence of hypoxanthine and guanine respectively.

Kinetic constant ...	$V_{\max}$ (munits/ml)						K_m (μ M)					
	A		H		G		A		H		G	
	Cysts	Nauplii	Cysts	Nauplii	Cysts	Nauplii	Cysts	Nauplii	Cysts	Nauplii	Cysts	Nauplii
Hypoxanthine			0.7	0.5					1	1		
Guanine					1.3	1.0					1	1
Adenine	12	12					3	2				
<i>PP</i> -Rib- <i>P</i> /Mg ²⁺ ratio	13	13	0.8	0.9	2.0	1.4	34	28	15	15	15	15
Mg ²⁺	10	10	0.8	0.8	1.7	1.4	180	150	40	50	45	50
Mn ²⁺	12	11	1.4	1.4	2.0	1.8	40	50	15	20	15	20
Zn ²⁺	11	8	0.8	0.8	1.6	1.7	50	45	66	75	75	75

were eluted by the addition of increasing concentrations of *PP*-Rib-*P* to the elution buffer: 0.2 mM for APRTase and 5 mM for HGPRTase. The elution profile of this chromatographic run is shown in Fig. 1. This step resulted in approx. a 2500-fold purification, with 30 % recovery of the enzymes in respect to the 100 000 g supernatant. The ratio of guanine to hypoxanthine activity, about 2:1, does not change during the purification procedure.

The *PP*-Rib-*P*-affinity-chromatography-purified enzymes, as well as those in the (NH₄)₂SO₄ preparations, were stable at -20 °C for at least 12 months. The purity of the final preparation was tested by denaturing SDS-PAGE. The migration as an apparent single protein band (Fig. 2) suggests that the enzymes have been essentially purified to homogeneity.

Physical properties

Some physical properties of the enzymes were determined, including measurements of the enzymes' behaviour on SDS-PAGE and Sephadex G-150 gel filtration.

The sub-unit structure of purified phosphoribosyltransferases was analysed by SDS-PAGE. As seen in Fig. 2, the purified proteins migrate as a single band. The subunit M_r of the *Artemia* phosphoribosyltransferases from cysts and nauplii were 15000 for APRTase and 19000 for HGPRTase, based on data obtained from the SDS-PAGE gradient (5–20 %). (Fig. 2). However, if the purified enzymes were treated for different periods at 100 °C under denaturing conditions in SDS, different molecular-mass products were obtained (results not shown). When cyst HGPRTase was incubated with SDS for 2, 4 and 10 min at 100 °C and run in 10 %-acrylamide gels, the M_r of the bands obtained ranged from 17000 to 69000 and appeared to be in multiples of 17000. After heat treatment for 2 min, a heavy band of M_r 69000 and two bands of M_r 53000 and M_r 17000 were observed, and treatment for 10 min yielded a single protein band of M_r 17000. This value of 17000 is in reasonable agreement with the M_r of 19000 obtained in the experiment shown in Fig. 2.

In gel-filtration chromatography (Fig. 3), the native M_r is estimated to be 66000 for HGPRTase, which is eluted in a single peak, and 28000 for APRTase. The same M_r values were obtained for the cyst and nauplii enzymes.

The effect of pH on the phosphoribosyltransferase activities from cysts and nauplii was studied using two buffer systems over a pH range 5.5–9.5 (Fig. 4). As pH increased from 5.5 to 8.0, the APRTase activity rate increased, reaching a maximum at pH 8.0

Table 3. Ca²⁺ inhibition of the APRTase from *Artemia* cysts

The purified APRTase was exhaustively dialysed against buffer A lacking Mg²⁺, and the enzyme activity determined as described in Materials and methods, adjusting in each case the metal concentrations. The data are expressed as a percentage of the APRTase activity in respect to the activity measured with Mg²⁺ and/or Mn²⁺ without Ca²⁺.

Cation added	Concn. (μ M)	[Ca ²⁺] (μ M)...	Inhibition (%)			
			0	25	50	400
Mg ²⁺	100		100	94	49	31
	200		100	91	67	47
	1000		100	96	76	85
Mn ²⁺	25		100	76	54	32
	50		100	75	65	46
	100		100	100	86	80

and decreasing thereafter. At the same pH, activity in imidazole was lower than that measured in Tris/HCl buffer. For HGPRTase, two points of pH optimum were determined, at pH 7.0 with both imidazole and Tris/HCl and at pH 8.5–9.0 with Tris/HCl. The enzymes show identical pH dependence with both hypoxanthine and guanine, and no differences in the regulatory properties and kinetics constants were observed at the two optimum pH points.

Bivalent-cation requirement and nucleotide effect

Mg²⁺ was essential for the activity of these enzymes, although the concentrations required for maximal velocity differed. Thus HGPRTase reached maximum activities at 1 mM, equimolar with the *PP*-Rib-*P* concentrations used, while APRTase showed maximum activities at twice the *PP*-Rib-*P* concentration (2 mM).

The functions of Mg²⁺ can be substituted equally or more effectively by Mn²⁺ and Zn²⁺ (Table 2).

At a 1 mM concentration, the activity with Ba²⁺ and Ca²⁺ is undetectable, and Ca²⁺ inhibited the enzyme activities by 50 % at 0.2 mM, even in the presence of 0.2 mM-MgCl₂ and/or 0.05 mM-MnCl₂ (Table 3).

The effect of purine nucleotides, even Gp⁴G and Gp³G, on enzyme activities was tested at saturated concentrations of *PP*-Rib-*P* and the corresponding bases, hypoxanthine, guanine and

Fig. 5. Double reciprocal plots of the APRTase (a), HPRTase (b) and GPRTase (c) activities

Initial velocities are given as a function of substrate concentrations. The effect of adenine, hypoxanthine and guanine concentrations at various fixed levels of *PP*-Rib-*P* is shown. Enzyme activities were measured as described in the Materials and methods section. The *PP*-Rib-*P* concentrations were: 10 μM (●), 20 μM (□), 50 μM (○), 100 μM (■), 200 μM (△), 1000 μM (■).

Fig. 6. Double-reciprocal plots of the HGPRTase activity as a function of substrate concentration: effect of *PP*-Rib-*P* concentrations at various fixed levels of guanine

Enzyme activities were measured as described in the Materials and methods section. The guanine concentrations were: 1 μM (●); 3.5 μM (×); 6 μM (○); 11 μM (□); 21 μM (△).

adenine. At a 1 mM-nucleotide concentration, only AMP had a significant inhibitory effect, about 80 % on the APRTase activity from both cysts and nauplii, while 1 mM-GMP showed approx. 70 % inhibition of hypoxanthine-guanine activities from both sources.

Kinetic properties

The Michaelis constants measured under our standard conditions and treated in terms of free concentrations are presented in Table 2. The K_m values for adenine of APRTase from cysts and nauplii were calculated as 3 and 2 μM respectively. Double-reciprocal plots of the reaction velocity as a function of *PP*-Rib-*P* concentration, when the ratio of Mg^{2+} to *PP*-Rib-*P* is held constant at 1:1 or 5:1, are linear, and optimal enzyme activity is obtained when the Mg^{2+} /*PP*-Rib-*P* ratio is approx. 2:1, with the apparent K_m for *PP*-Rib-*P* in these conditions being about 30 μM . The K_m values of HGPRTase for hypoxanthine and guanine are < 1 μM , while the K_m for *PP*-Rib-*P* is 15 μM . The apparent cation K_m values, at saturated levels of the substrates, are also listed in Table 2. Under these conditions, no appreciable differences are observed in the affinity of the phosphoribosyltransferases studied for Mg^{2+} , Mn^{2+} and Zn^{2+} . The only exception is the highest K_m value of adenine phosphoribosyltransferase for Mg^{2+} , which is approx. threefold that of the other enzymes.

Double-reciprocal plots of initial velocity against the concentration of the base substrate at a series of fixed concentrations of the second substrate, *PP*-Rib-*P*, are presented in Fig. 5. It can be seen that, at higher concentration of guanine and hypoxanthine in the HGPRTase reaction, the lines curve upwards in the graph, indicating substrate inhibition by the corresponding bases, with a K_i of 80 μM for hypoxanthine, and about 10 μM for guanine. Although no clear substrate inhibition by adenine was observed in the APRTase reaction, even up to 200 μM -adenine, methodological difficulties make impossible to test higher adenine concentrations. The inhibition by guanine is, apparently, not competitive with *PP*-Rib-*P* (Fig. 6).

While a very close similarity was observed in most of the kinetic properties of the phosphoribosyltransferases described above, the enzymes studied differ in their sensitivity to inhibitors

Fig. 7. Inhibition of *Artemia* APRTase activities by AMP; (a) cysts and (b) nauplii

The standard assay was utilized with adenine held constant at 52 μM. *PP-Rib-P* concentration was varied at 10–100 μM, and the MgCl₂ was in tenfold excess of *PP-Rib-P* in all cases. The AMP concentrations were: zero (□); 0.1 mM (○); 1.0 mM (Δ); 2 mM (●). The insets show the competitive inhibition with respect to *PP-Rib-P* with a K_i of approx. 100 μM. *PP-Rib-P* concentration: 0.05 mM (▲); 0.1 mM (■); 1.0 mM (×).

as well as in the mechanism of inhibition by reaction products. The effect of AMP and GMP on the initial rate of APRTase and HGPRTase reactions, at varying concentrations of each substrate in the presence of saturated concentrations of other substrates, was determined. No significant inhibition with respect to the corresponding base was detected, even at a 1 mM-nucleotide concentration. However, inhibition of APRTase by AMP is apparently competitive with respect to *PP-Rib-P*, with a K_i of 100 μM (Fig. 7), while GMP shows a mixed inhibition with *PP-Rib-P* on hypoxanthine HGPRTase activity with a K_i of 150 μM (Fig. 8). Similar results were observed with guanine HGPRTase. IMP did not significantly inhibit HGPRTase, even at a 1 mM concentration.

DISCUSSION

APRTase and HGPRTase have been purified 2500-fold from *Artemia* cysts and nauplii from the 100 000 *g* supernatant, to apparent homogeneity. The method described here has some important advantages over previously reported purification pro-

Fig. 8. Inhibition of *Artemia* HGPRTase activities with hypoxanthine by GMP; (a) cysts and (b) nauplii

The standard assay was utilized with hypoxanthine held constant at 4 μM and the *PP-Rib-P* and MgCl₂ as described in Fig. 7. The GMP concentrations were: zero (□); 0.1 mM (○); 1.0 mM (Δ); 2 mM (●). The *PP-Rib-P* concentrations in the insets were: 0.01 mM (▲); 0.1 mM (■); 1.0 mM (×). The mixed inhibition shows a K_i of 150 μM.

cedures [21,22] as regards reproducible high purity and greater yields, so that much time is saved. The procedure differs from our former published protocol [12] in that the enzymes were obtained from the high-speed supernatant fraction, followed by (NH₄)₂SO₄ fractionation and one affinity-chromatography step, Sepharose-AMP, which adsorbs both proteins. The activities are eluted by the addition of increasing *PP-Rib-P* concentrations to the elution buffer; 0.2 mM and 5.0 mM-*PP-Rib-P* are required for APRTase and HGPRTase elution respectively.

Each of the purified enzymes from both sources exhibit one protein band on SDS-PAGE and one major activity peak on Sepharose G-150 gel filtration, which correspond to APRTase and HGPRTase proteins. The enzymes are stable under storage conditions in the corresponding elution buffer at -20 °C for a minimum period of 12 months, with negligible loss of activity. The final specific activity of the APRTases were 13000 and 14000 munits/mg for cysts and nauplii, and the HGPRTases 500 and 650 munits/mg with hypoxanthine and 1000 and 1300 munits/mg in the presence of guanine.

The ratio of the activities with guanine to hypoxanthine, approx. 2:1, changed only slightly during the purification of the

enzymes [22]. This ratio value differs from those reported for other parasitic-protozoan and human enzymes [23–26] and, unlike most purine phosphoribosyltransferases, the APRTase activities are 10–20-fold higher than those determined for hypoxanthine-guanine phosphoribosyltransferases. These results explain the greater incorporation rate of [^{14}C]adenine by *Artemia* nauplii with respect to [^{14}C]hypoxanthine and [^{14}C]guanine uptake [12].

The subunit M_r of the APRTase enzymes, as determined by SDS-PAGE, is approx. 15000. The native M_r values were estimated to be about 28000, indicating that the enzymes are dimers, while the native HGPRTase proteins have an M_r of 66000 and a subunit structure consisting of four identical polypeptide chains with an apparent M_r of 18000–19000. The native M_r reported for the phosphoribosyltransferase enzymes from different sources are in the range 20000–40000 [22,27–29] for APRT and 60000–100000 for HGPRT [21,22,25,30]. Moreover, the M_r and subunit structure of these enzymes are a matter of controversy, especially as regards the HGPRT. This enzyme has been proposed to exist as a dimer [25,30,31], trimer [21,32–34] and tetramer [21–25,35] of identical sub-units.

The differences encountered in the M_r and number of sub-units could be due to experimental artefact, since we have detected differences in the number and size of the subunits in our preparations, depending on SDS treatment time, which might explain the diversity of data found in the documentation on this subject.

The kinetic constants for the phosphoribosyltransferases were determined and found to be similar to those of the enzymes for other sources [21,36]. The low K_m for adenine, hypoxanthine and guanine are typical of many phosphoribosyltransferases. The K_m values for *PP-Rib-P*, also similar to that described, were determined to equimolar Mg^{2+} concentration and with free Mg^{2+} in the reaction assay. No important differences were found, although the optimum $V_{\max}$ is reached with the ratio *PP-Rib-P*/ $\text{Mg} = 1:2$. The K_m for the bivalent cations Mg^{2+} , Mn^{2+} and Zn^{2+} were determined under saturating concentrations of *PP-Rib-P* and the corresponding base.

The kinetics of the enzyme activities and its dependence on the concentration of the bases at different fixed concentrations of *PP-Rib-P* are compatible with substrate inhibition of HGPRTases by hypoxanthine and guanine. This type of phosphoribosyltransferase inhibition by substrate only has been described by Steyn & Harley in an HGPRTase from a patient with moderate enzyme deficiency [26].

Previous studies have established the requirement for metal ions during the course of phosphoribosyltransferases reactions [37–39], with variation in the requirement for activation by cations. The Mg^{2+} cation is generally used; however, this may be generally substituted by Mn^{2+} , and by Co^{2+} and Zn^{2+} in yeast [37–40].

Calcium ion inhibition has been reported by Hochstadt-Ozer & Stadtman [28] in the *E. coli* APRTase enzyme, and by Hochstadt in enteric bacteria HGPRTase [41]. From the point of view of the metal effector, the *Artemia* enzymes are similar to those of lower organisms [28,40]. They are activated by Mg^{2+} , Mn^{2+} and Zn^{2+} , and inhibited by Ca^{2+} , even in the presence of high Mg^{2+} concentrations.

No differences have been found in the properties and levels of the phosphoribosyltransferases examined in the different stages of *Artemia* development. Neither the physical chemistry nor the kinetic properties differ significantly between *Artemia* and other systems, although they are more similar to the phosphoribosyltransferases of lower organisms. Previous studies with *Bacillus subtilis* [42] suggest that purine pyrophosphate phosphoribosyltransferases play a role in regulating the uptake of different

purines, being proportional to the relative activity of the phosphoribosyltransferases [3,4]; however, the precise correlation between base intake and enzyme properties which have been described in *E. coli* and *Schizosaccharomyces* [3,4] does not exist in *Artemia*.

The changes in the intake of adenine, hypoxanthine and guanine by *Artemia* nauplii observed *in vivo* [12] do not reflect exactly the kinetic behaviour of the corresponding phosphoribosyltransferases *in vitro*. While the specific activities of the enzymes do not change through larval development, the uptake of purine bases increases with increasing developmental time, about 7-fold between immediately post-hatching cysts and 30-h nauplii (P. Rotllan, A. Liras, & P. Llorente, unpublished results).

The substrate inhibition by guanine observed in the HGPRTase reaction, could indicate a form of intracellular control of the possible high concentrations of the base, at least in the vicinity of the active site.

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