Two isozymes of the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) of the apicomplexan protozoan Toxoplasma gondii are encoded by the single HGPRT gene as a result of differential splicing. Western blotting of total T. gondii protein shows that both isozymes I and II, which differ by 49 amino acids, are expressed. Both form enzymatically active homotetramers when overexpressed in Escherichia coli. The specific activity of HGPRT-I is five times that of HGPRT-II. When both isozymes are co-expressed in E. coli, HGPRT-I/HGPRT-II heterotetramers form. The predominant heterotetramer has enzymatic activity similar to that of HGPRT-II, and gel filtration chromatography demonstrates that its size is intermediate between the sizes of HGPRT-I and HGPRT-II. Mass spectrometric analysis of cross-linked homo- and heterotetramers reveals species of distinct molecular mass for HGPRT-I, HGPRT-II, and HGPRT-I/HGPRT-II and suggests that the predominant heterotetramer consists of one HGPRT-I subunit and three HGPRT-II subunits. The implications of this finding are discussed.

Encephalitis caused by the apicomplexan protozoan Toxoplasma gondii is the second leading cause of death among patients with AIDS (1). Much effort has been expended on the development of new drugs to treat T. gondii infections (2, 3). A widely recognized drug target in parasitic protozoa is the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT1; EC 2.4.2.8) (4, 5). HGPRT catalyzes the Mg2+-dependent conversion of hypoxanthine, guanine, or xanthine and 5-phosphoribosyl-1-pyrophosphate (PRPP) to purine nucleotides and inorganic pyrophosphate. It is a key purine salvage enzyme required for growth and replication (6, 7).

The cloning of T. gondii HGPRT revealed the presence of two cDNAs, differing by 147 nucleotides, that appear to result from differential splicing of the nascent transcript from the single HGPRT gene (8, 9). It is the smaller 230-amino acid isozyme (I), which is homologous to human HGPRT, that we (11, 12) and others (13) have crystallized. Enzymatically active HGPRT-I is a homotetramer (12). The larger T. gondii HGPRT isozyme, HGPRT-II, has a 49-amino acid insertion following Gln7 (GenBank™ accession number U10083). How the three-dimensional structure of HGPRT-I is altered to accommodate the insertion in HGPRT-II, which is located at a subunit interface within the HGPRT tetramer, is not known.

T. gondii HGPRT is the only HGPRT known that may exist as two isozymes. From a drug design perspective, therefore, it is important to know whether Toxoplasma expresses HGPRT-II as a stable enzyme, and if so whether HGPRT-I or HGPRT-II (or both) is the true drug target in Toxoplasma. If both HGPRT-I and HGPRT-II are expressed in Toxoplasma, do they form homotetramers only, or are they capable of forming heterotetramers as well? If so, do they form a preferred heterotetramer or a statistical mixture of all possible heterotetramers? How do the enzymatic activity and sensitivity to inhibitors of HGPRT heterotetramers compare with HGPRT-I and HGPRT-II homotetramers?

We report here our initial investigations into these questions, which show that T. gondii does express stable HGPRT-II, that the HGPRT-II heterotetramer possesses enzymatic activity distinct from that of HGPRT-I, that HGPRT-I and HGPRT-II form a particular heterotetramer when both are co-expressed in Escherichia coli, and that this heterotetramer possesses enzymatic activity similar to that of the HGPRT-II homotetramer.

**EXPERIMENTAL PROCEDURES**

**HGPRT Expression**

Subcloning into pET9a—Plasmids pET15b-C1 (previously pETC1; Ref. 8) and pET15b-C3 (pETC3; Ref. 8), which contain the T. gondii HGPRT isozymes I and II coding sequences, respectively, inserted into vector pET15b (Novagen, Inc.; Ref. 14), were digested with NdeI and BglII overnight at 37 °C. pET9a was digested with NdeI and BamHI overnight at 37 °C. pET9a was digested as well. pET9a is similar to pET15b but lacks the N-terminal His6 tag and contains a kanamycin resistance marker. Digests were separated by electrophoresis on a 1% agarose gel; the appropriate fragments were extracted from the gel (GeneClean, Bio 101, Inc.) and then ligated with T4 DNA ligase (16 h, 15 °C). Competent E. coli XL1 Blue cells were transformed to kanamycin resistance with the ligated DNAs and grown on 2x-XT (16 g/liter tryptone, 10 g/liter yeast extract, and 5 g/liter NaCl) agar plates containing 25 μg/ml kanamycin. Colonies were screened for the presence of HGPRT-I and HGPRT-II inserts by restriction enzyme (NdeI and BamHI) analysis of plasmid DNA. Two positive clones were named pET9a-C1 and pET9a-...
C3. Open reading frames in each plasmid construct were confirmed by automated DNA sequencing (PRISM Dye Terminator kit/PRISM 377 sequencer, Applied Biosystems, Inc.) to ensure that no sequence artifacts were introduced during the subcloning process.

**Co-expression of HGPRT-I and HGPRT-II—**Competent *E. coli* BL21(DE3)/pET9a-C1 and BL21(DE3)/pET15b-C3 were separately inoculated in H2O. The band at 37 °C with 0.1% Coomassie Brilliant Blue G-250, destained, and rinsed with ice and was followed by passage through a small benzamidine-agarose affinity column to remove the thrombin. Additional protease inhibitors were added to the purified enzyme. *T. gondii* HGPRT heterotetramers were purified from the co-expression cultures in the same manner. HGPRT kinetics were measured spectrophotometrically at 37 °C as described (12), in a buffer containing the purine base, PRPP, 100 mM Tris-HCl, pH 8.0, 20 mM MgCl₂, 0.1 mM EDTA, and 0.1 mg/ml bovine serum albumin. *V*ₘₐₓ values were converted to *kₐₜₐₜ* values using protein concentrations determined by Bradford assay (bovine γ globulin standard, Ref. 15).

**Subunit Ratio and Cross-linking—**Discontinuous SDS-polyacrylamide gel electrophoresis (PAGE), performed according to Laemmli (16), was used to assess HGPRT purity, to determine approximate subunit molecular masses, and to assess the extent of cross-linking reactions. HGPRT-I to HGPRT-II ratios were determined by densitometry (Shimadzu CS-9000 dual wavelength scanning, with computer software, single lane scans at 595 nm) of gels stained with Coomassie Brilliant Blue G-250 (Pierce Gelcode Blue). Cross-linking reactions were performed by incubating 100 µl of HGPRT (2.5 mg/ml) in phosphate-buffered saline and 2 µl of diisuccinimidyl suberate (20 mM in Me2SO, Pierce) for 30 min at room temperature. Reactions were quenched by addition of 1 µl of 1 M ethanolamine.

**Western Blotting of *T. gondii* Total Soluble Protein—**Purified recombinant *T. gondii* HGPRT-I was separated on 12% SDS-PAGE, stained with 0.1% Coomassie Brilliant Blue G-250, destained, and rinsed extensively in H₂O. The band at ~27 kDa was cut from the gel. Polyclonal rabbit antisera were prepared by HRP Inc. (Denver, PA). The minced gel containing ~125 µg of protein was injected subcutaneously into each of two rabbits along with Freund’s adjuvant. Two additional injections were made, and the rabbits were bled for serum production. Preeimmunization bleedings were also taken. *T. gondii* parasites (RH strain tachyzoites) were lysed by freeze-thawing and brief sonication in 25 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 1 mM PRPP, 1% Nonidet P-40. The lysate was centrifuged (10,000 g, 4 °C, 10 min). 12 µg of *T. gondii* soluble protein was separated by 12% SDS-PAGE, recombiant *T. gondii* HGPRT-I and HGPRT-II (400 ng each) and molecular mass markers were included on the gel as standards. The gel was transferred to Hybond-ECL (Amersham Pharmacia Biotech) in Towbin buffer (25 mM Tris-HCl, 192 mM glycine, 20% (v/v) methanol, pH 8.3, at 100 V for 1 h). A 1:100 dilution of the rabbit polyclonal anti-HGPRT-I antiserum was used as the primary antibody, and a 1:1500 dilution of the Enhanced Chemiluminescence Western Kit (Amersham Pharmacia Biotech) anti-rabbit IgG was used as the secondary antibody. Preeimmunization serum was used in control experiments.

**Gel Filtration Chromatography—**Samples were applied at 4 °C to a HiLoad 26/60 Superdex 200 gel filtration column (Amersham Pharmacia Biotech) equilibrated with 25 mM Tris-HCl, pH 8.0, 10 mM MgCl₂, 100 mM KCl, 1 mM diethylenetriol, 0.1 mM EDTA, and 10% glycerol. The column was calibrated with thyroglobulin (Stokes radius, 85.0 Å), dolase (48.1), ovalbumin (30.5), and ribonuclease A (16.4). Partition coefficients were calculated from the elution volumes of the proteins, blue dextran (~2 × 10⁶ Da), and acetone. Stokes radii were determined from partition coefficients using an empirical inverse error function complement (erfc⁻¹) relationship (17). Frictional coefficients and Perrin shapes were calculated by using the second moment of the Stokes equation (18).

**Mass Spectrometry and Amino Acid Sequencing—**MALDI-TOF mass spectra were obtained on a Voyager Elite mass spectrometer (positive mode) with delayed extraction technology (PerSeptive Biosystems). The acceleration voltage was set at 25 kV, and 10–50 laser shots were summed. The matrix was sinapinic acid (Aldrich) dissolved in CH₃CN/0.1% CF₃COOH (1:1). The mass spectrometer was calibrated with a recombinant bovine α-globin or bovine serum albumin. Samples were diluted 1:10 with matrix before pipetting 1 µl onto a smooth plate. N-terminal sequencing was done by automated Edman degradation on a gas-phase microsequencing system (model PI 2090E, Beckman). The amino acid residue released in a given cycle was identified from the difference chromatogram (comparison with the previous cycle).

**RESULTS**

**Expression, Purification, and Characterization of HGPRT-II—**We showed previously that expression of *T. gondii* HGPRT-I in *E. coli* (pET15b vector) provides large quantities of homogeneous, enzymatically active protein (12). HGPRT-II was expressed using the same approach. It was purified to apparent homogeneity by Ni²⁺-agarose affinity chromatography, proteolytic removal of the N-terminal His₆ tag, and gel filtration chromatography. Isozyme II had enzymatic properties similar to but distinct from isozyme I. As shown in Table I, HGPRT-II had about one-fifth the specific activity of HGPRT-I regardless of whether hypoxanthine, guanine or xanthine was the substrate. The Michaelis constants of the two isozymes were similar, with the PRPP *Kₘ* of HGPRT-II tending to be higher than that of HGPRT-I. Also, the guanine *Kₘ* of HGPRT-II was six times higher than that of HGPRT-I, with the result that the catalytic efficiency of the isozymes differed by 30-fold with this substrate. These kinetic differences between HGPRT-I and HGPRT-II agree in broad terms (except for guanine) with those reported previously (9).

The subunit molecular mass of HGPRT-II, as determined by SDS-PAGE, was 33,500 Da. Both it and HGPRT-I (27,500 Da) migrated at a somewhat higher position than that predicted from their calculated molecular masses. Nonetheless, mass spectrometric analysis (MALDI-TOF) of both isozymes confirmed that they possessed the correct mass (for HGPRT-I, the observed mass was 26,668 Da and the calculated mass was 26,676 Da; for HGPRT-II, the observed mass was 31,767 Da and the calculated mass was 31,766 Da; masses include an N-terminal peptide, Gly-Ser-His, that remains after thromboytic removal of the His₆ tag).

**HGPRT-II eluted much earlier than HGPRT-I from a gel filtration column, well in excess of that expected from molecular mass differences, suggesting that it possessed a higher oligomeric state than HGPRT-I (Fig. 1). Comparison of its elution volume with those of standards of known size revealed that HGPRT-II had a Stokes radius of 49.2 Å, compared with 38.2 Å for HGPRT-I (17). We had determined previously from its Stokes radius that HGPRT-I was a tetramer (Perrin shape factor *F*, 1.04; axial ratio, ~1.91; assumes 0.35 g of H₂O/ρg of HGPRT-I), a result that has been verified repeatedly by the observation of this aggregation state exclusively in more than eight distinct *T. gondii* HGPRT crystal structures (axial ratio ~1.61; Refs. 11–13). When the shape factor was calculated for possible HGPRT-II aggregation states (dimer through octamer), only an octamer appeared physically reasonable (d: 3.5:1.9:1.6:1; Refs. 11–13). When the shape factor was calculated for possible HGPRT-II aggregation states (dimer through octamer), only an octamer appeared physically reasonable (d: 3.5:1.9:1.6:1; Refs. 11–13). When the shape factor was calculated for possible HGPRT-II aggregation states (dimer through octamer), only an octamer appeared physically reasonable (d: 3.5:1.9:1.6:1; Refs. 11–13).
T. gondii HGPRT Heterotetramers

Enzymatic activity was determined at pH 8.0, 37 °C. \( K_m \) values are reported for the varied substrate; the concentration of the other substrate was fixed at ten times its \( K_m \) value.

### TABLE I

*Enzymatic activity of the T. gondii HGPRT isozymes*

| Enzyme                                                                 | \( k_{cat} \) | \( K_m \) (μM) Hypoxanthine | \( K_m \) (μM) PRPP | \( k_{cat}/K_m \) Hypoxanthine | \( k_{cat}/K_m \) PRPP |
|------------------------------------------------------------------------|---------------|-----------------------------|-------------------|-------------------------------|------------------------|
| **HPR-T reaction**                                                     |               |                             |                   |                               |                        |
| HGPRT-I                                                                | 7.59          | 1.6 ± 0.6                    | 42.9 ± 4.2        | 4.63                          | 0.177                  |
| HGPRT-II                                                               | 1.74          | 1.4 ± 0.2                    | 75.4 ± 7.3        | 1.20                          | 0.023                  |
| HGPRT-I/HGPRT-II heterotetramer*                                        | 1.96          | N.D.                         | N.D.              | N.D.                          | N.D.                   |
| **GPR-T reaction**                                                     |               |                             |                   |                               |                        |
| HGPRT-I                                                                | 12.7          | 2.1 ± 0.7                    | 74.6 ± 20.0       | 6.02                          | 0.170                  |
| HGPRT-II                                                               | 2.46          | 11.8 ± 1.4                   | 92.1 ± 10.5       | 0.207                         | 0.027                  |
| HGPRT-I/HGPRT-II heterotetramer*                                        | 3.66          | N.D.                         | N.D.              | N.D.                          | N.D.                   |
| **XPR-T reaction**                                                     |               |                             |                   |                               |                        |
| HGPRT-I                                                                | 31.0          | 14.4 ± 1.7                   | 146 ± 35          | 2.15                          | 0.213                  |
| HGPRT-II                                                               | 6.86          | 16.2 ± 1.1                   | 270 ± 31          | 0.424                         | 0.025                  |
| HGPRT-I/HGPRT-II heterotetramer*                                        | 7.96          | N.D.                         | N.D.              | N.D.                          | N.D.                   |

* HGPRT-II-tagged. The Ni\(^{2+}\)-agarose affinity-purified heterotetramer was further purified by gel filtration chromatography (Fig. 1, bottom panel).

* Based on comparative specific activity measurements of HGPRT-II and the HGPRT-I/HGPRT-II heterotetramer.

* N.D., not determined.

HGPRT-II either adopts a dramatically more open conformation than HGPRT-I, or more likely that it, like HGPRT-I, forms a tetramer, but one prone to further aggregation to an octamer under the chromatography conditions.

*Both HGPRT-I and HGPRT-II Are Expressed in Toxoplasma—*Immunological detection was used to determine whether both *T. gondii* HGPRT cDNAs give rise to stable protein in the parasite. A polyclonal rabbit antiserum was raised against homogeneous recombinant HGPRT-I. Western blotting showed that the antiserum cross-reacted well with recombinant HGPRT-I, even when the relative expression levels of HGPRT-I and HGPRT-II differed. This is the first evidence that HGPRT heterotetramers can and do form when both isozymes are heterologously expressed in *E. coli*. Control experiments showed that untagged HGPRT-I, HGPRT-II, or HGPRT-I/HGPRT-II heterotetramers, by themselves, do not bind to Ni\(^{2+}\)-agarose.

When HGPRT-II bore the His\(_6\) tag, both proteins were expressed at moderate, approximately equal levels (Fig. 3, top panel). The majority of the HGPRT bound specifically to Ni\(^{2+}\)-agarose as the 1:3 HGPRT-I/HGPRT-II heterotetramer. Excess untagged HGPRT-I, which did not bind, was enzymatically active. The purified heterotetramer had specific enzymatic activity much more similar to HGPRT-II than HGPRT-I (Table I).

In the reciprocal experiment, tagged HGPRT-I was barely detectable in cell lysates 3 h after isopropyl-\( \beta \)-D-thiogalactopyranoside induction, whereas untagged HGPRT-II was expressed at very high levels (Fig. 3, bottom panel). As before, the 1:3 HGPRT-I/HGPRT-II heterotetramer bound specifically to Ni\(^{2+}\)-agarose, whereas the large excess of untagged HGPRT-II did not. Unbound co-expressed HGPRT-II appeared to have a lower molecular mass by SDS-PAGE (31,500 Da) compared with HGPRT-II expressed alone (33,500 Da, after thrombin cleavage of the His\(_6\) tag). Purification of unbound HGPRT-II by gel filtration chromatography (Stokes radius, 50.2 Å) provided a sample of >95% purity that possessed enzymatic activity equivalent to that of HGPRT-II expressed alone. N-terminal sequencing (ASKPIEESR) and MALDI-TOF mass spectrometry (observed mass was 31,408 Da) suggested that it comprised amino acids 2–279 of HGPRT-II (calculated mass was 31,353 Da).
It is not clear why HGPRT-II lacking the N-terminal 4-residue remnant (GSHM) of the His tag migrates at the expected position by SDS-PAGE, whereas HGPRT-II and HGPRT-I with the tag remnant migrate at slightly higher than expected masses. SDS-PAGE yields at best only approximate molecular masses, because of its sensitivity to a variety of factors. Nevertheless, the mass spectral results for all three proteins agree very well with the masses calculated from their amino acid compositions.

Additional Evidence That HGPRT-I and HGPRT-II Heterotetramers Form—Although the results of the co-expression experiments presented above, by their very design, provided strong evidence that HGPRT heterotetramers were formed, we sought additional confirmatory evidence. As shown in Fig. 1, the Ni\textsuperscript{2+}-agarose-bound HGPRT heterotetramer had a gel filtration elution volume more similar to the elution volume of HGPRT-II than HGPRT-I. A control experiment with a physical mixture of the two isozymes (Fig. 3) showed conclusively that the peak of intermediate mobility was due to a distinct heterotetrameric species (Stokes radius, 47.7 Å).

Cross-linking experiments were also performed on HGPRT-I, HGPRT-II, a physical mixture of HGPRT-I and HGPRT-II, and the co-expressed, purified HGPRT-I\textsubscript{z}HGPRT-II (peak from the gel filtration column; Fig. 1, bottom). Cross-linking with disuccinimidyl suberate was confirmed by SDS-PAGE and MALDI-
T. gondii HGPRT Heterotetramers

Cross-linked HGPRTs were analyzed by MALDI-TOF mass spectrometry. The mass calculated from the subunit composition is compared with that determined by mass spectroscopy.

| Oligomeric state | Calculated mass | Observed mass | Difference in mass | Fig. 4 peak |
|------------------|-----------------|---------------|-------------------|-------------|
| Dimer            | Da              | Da            | Da                | C           |
| II-II            | 63,532          | 64,281        | 749               |             |
| II-II'           | 60,647          | 61,523        | 876               | B           |
| II-I             | 58,021          | 58,720        | 699               | A           |
| II'-I            | 57,762          | N.O.          | N.O.              |             |
| I-I              | 55,136          | N.O.          | N.O.              |             |
| II'-II           | 52,510          | N.O.          | N.O.              |             |
| Trimer           |                 |               |                   |             |
| II-III           | 95,298          | 96,413        | 1,115             | F           |
| II-III'          | 92,413          | 93,609        | 1,196             | E           |
| II-I             | 89,787          | 90,805        | 1,018             | D           |
| II'-I             | 89,528         | N.O.          | N.O.              |             |
| II'-II            | 86,902         | N.O.          | N.O.              |             |
| II'-II'           | 86,643         | N.O.          | N.O.              |             |
| II-I               | 84,576         | N.O.          | N.O.              |             |
| II'-I              | 84,017         | N.O.          | N.O.              |             |
| II'-II            | 81,391         | N.O.          | N.O.              |             |
| I-I                 | 78,765         | N.O.          | N.O.              |             |

*Calculated subunit mass based on the amino acid composition: HGPRT-II (II), 31,766 Da [tagged, thrombin-cleaved, GSH

\[\text{HGPRT-II: } (\text{II}), \quad 28,881 \text{ Da (tagged, thrombin-cleaved, and further proteolyzed, } \text{CTPE...)} \].

Cross-linked HGPRT-I: (II), 25,265 Da [tagged, thrombin-cleaved, \(2\text{ASKPIE...}\)].

Calculated mass. Includes experimental error as well as mass attributable to covalently attached cross-links and ethanolamine-quinched cross-link (+138 and +199 Da, respectively).

N.O., not observed. The expected positions of the HGPRT-I dimer and trimer are marked with stars in Fig. 4.

The mass spectrum of cross-linked HGPRT-I:HGPRT-II (HGPRT-I untagged, observed subunit mass, 26,218 Da; calculated mass (residues 2–230), 26,255 Da; HGPRT-II tagged and thrombin-cleaved, subunit mass, 31,667 Da) is notable for the absence of cross-linked HGPRT-I peaks (Fig. 4, left star). By contrast, a new peak for the HGPRT-I/HGPRT-II heterodimer was observed (peak A). Peaks for the HGPRT-II/HGPRT-I homodimer and the HGPRT-II-HGPRT-II' heterodimer were also observed. Similarly, of the possible cross-linked trimers, only those corresponding to the HGPRT-II homotrimer and the HGPRT-II/HGPRT-II/HGPRT-II' and HGPRT-II/HGPRT-II/HGPRT-II'/HGPRT-II homotetramer were observed. The expected HGPRT-I homotrimer was not observed (Fig. 4, right star). The simplest interpretation of these results is that co-expressed HGPRT consists of a tetramer that contains no more than one HGPRT-I subunit, i.e. a 1:3 HGPRT-I-HGPRT-II heterotetramer.

**DISCUSSION**

HGPRT has been characterized from many mammalian, protozoal, and bacterial organisms. To the best of our knowledge, only the HGPRT from *T. gondii* has been found to exist as two isoforms. This finding is especially interesting for two reasons. First, *T. gondii* HGPRT is a novel drug target for the treatment of opportunistic toxoplasmosis in AIDS patients (4, 5). Because there are two HGPRTs in *T. gondii*, both should be evaluated as drug targets. Second, the two isoforms, HGPRT-I and HGPRT-II, are encoded by a single HGPRT gene (9). Two research groups each isolated from *T. gondii* cDNA libraries two mature HGPRT transcripts that appear to result from differential
splicing of the nascent transcript (8, 9). We thought at first that the isolation of the HGPRT-II cDNA was a cloning artifact, perhaps as a result of inefficient or incomplete processing of the nascent HGPRT-II mRNA to the mature, HGPRT-I mRNA in T. gondii. This thought was reinforced by the fact that it is the smaller HGPRT-I that is homologous to all other known HGPR Ts, including its closest known relative, Plasmodium falciparum HGPRT (19, 20), and that all other organisms possess only one HGPRT protein.

Our experiments with the HGPRT-II isozyme show that it possesses high enzymatic activity when expressed in E. coli, like HGPRT-I. Donald et al. (9) previously reported enzymatic activities for recombinant HGPRT-I and HGPRT-II that agree relatively well with our values (Table I). Because of purification difficulties with HGPRT-II, however, these workers were not able to state conclusively whether the differences they observed between HGPRT-I and HGPRT-II were real. Our straightforward purification of HGPRT-II now shows that it is indeed enzymatically different from HGPRT-I.

Western blotting of T. gondii total soluble protein, using polyclonal antiserum raised against recombinant HGPRT-I, demonstrated that both isozymes are expressed as protein in the parasite. Our findings indicate, therefore, that the isolation of two HGPRT cDNAs from T. gondii was not an artifact but rather that this organism actually has two functional HGPR Ts. Although it is not clear why Toxoplasma has two HGPR Ts, the significance for drug design is clear: recombinant HGPRT-I and HGPRT-II would also be unprecedented. Analitical ultracentrifugation and crystallization experiments with HGPRT-II and the heterotetramer are planned to address this issue. Finally, further studies are contemplated to express tagged HGPRT-I and HGPRT-II in T. gondii itself, which will allow for the confirmation that heterotetramers form in situ. If so, their purification and characterization will be undertaken.

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The Two *Toxoplasma gondii* Hypoxanthine-Guanine Phosphoribosyltransferase Isozymes Form Heterotetramers

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