main cohesive energy to the thorium-hydrogen bonds.

The mean Th-H (terminal) and Th-H (bridging) bond distances in the dimer are 2.03(1) and 2.29(3) Å, respectively. The first value is close to the sum of covalent radii for hydrogen (0.30 Å) and thorium (1.79 Å, assumed equal to the metallic radius), and the second value is ~0.2 Å longer, as found for bridging hydrogen atoms in other transition metal complexes (*23*). For comparison, the thorium-hydrogen bonding distances in ThH_2 and Th_4H_{15} range from 2.29 to 2.46 Å. These longer distances can be attributed to the fact that the hydrogen atoms are more highly coordinated in the binary hydrides (three- and four-coordinated) than in the dimer (one- and two-coordinated).

Other important mean distances and angles in the structure are Th-C (ring), 2.83(1) Å; C (ring)-C (ring), 1.43(1) Å; C (ring)-C (methyl), 1.50(1) Å; C-H, 1.05(1) Å; and $(CH_3)_5C_5$ (centroid)-Th-$(CH_3)_5C_5$ (centroid), 130(1)°. Details of the structure will be published elsewhere (*24*).

Although this is the largest structural problem we have solved to date, we are routinely solving the structures of other organometallic compounds by direct methods with neutron diffraction data. Furthermore, the structural solution of the organic compound melampodin, which is noncentrosymmetric ($P2_12_12_1$) and contains 216 atoms per unit cell ($\Sigma b_H^2/\Sigma b^2_{all} = 0.219$), has been successfully obtained by application of the MULTAN computer program to neutron diffraction data (*25*). Thus we conclude that even for very large hydrogen-containing molecules, direct methods are a very powerful tool for solving structures in neutron diffraction studies.

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Human and Mouse Hypoxanthine-Guanine Phosphoribosyltransferase: Dimers and Tetramers

Abstract. *Human and mouse hypoxanthine-guanine phosphoribosyltransferase subunits combine to form an active heteropolymer. Dimers form the basic subunit structure of the enzymes, yet the dimers can readily associate to form tetramers. The equilibrium between dimers and tetramers is significantly influenced by the ionic strength of the enzyme solvent.*

Hypoxanthine-guanine phosphoribosyltransferase (HGPRT; E.C. 2.4.2.8.), the enzyme that catalyses the conversion of hypoxanthine and guanine to mononucleotides, is specified by mammalian X chromosomes (*1*, *2*). This enzyme has been the principal focus for studies of mutagenesis in mammalian cells (*3*, *4*); yet, neither the subunit structure nor the nature of interspecific subunit interactions have been defined. On the basis of estimates of the molecular weight of the purified enzymes from Chinese hamster, mouse, and human cells, it has been proposed that the enzymes are trimers (*5*). However, studies of interspecific hybrids (*6*) suggest that the enzymes may not be trimers, although these observations have been difficult to evaluate because of the presence of multiple enzyme bands in the parent cells as well as in hybrid cells. The analysis is complicated by the fact that HGPRT can exist in more than one form within a cell, as isoenzymes (*7*). Furthermore, the complexity is compounded by the presence of pseudoisoenzymes resulting from disulfide bond formation (*8*).

We have examined the interaction of the mouse and human enzyme subunits in hybrid cells, using conditions which eliminate pseudoisoenzymes (*8*). Our results indicate that the subunits of human and mouse HGPRT interact to form a single active heteropolymer and that, under the conditions of isoelectric focusing, the enzymes are dimers. Evidence is also presented that the enzyme dimers associate to form tetramers in solvents of high ionic strength.

Our strategy was to select hybrids on the basis of complementation at the thymidine kinase (TK) locus, and to look for those which retain a human X chromosome so that both murine and human forms of HGPRT are present in the hybrid cell. The hybrids were derived from normal human skin fibroblasts and LM (TK^-) Cl-1D, a derivative of the mouse L cell lacking TK but wild type for HGPRT. Fusion was mediated with 50 percent polyethylene glycol (*9*) in saline. Selection for the mouse/human hybrids was carried out in HOT medium [HAT (*3*) containing $10^{-6}M$ ouabain], which eliminates both parental cells. After 5 weeks of selection, hybrid clones were picked with stainless steel cylinders and transferred to individual dishes containing HOT. The clones were then replicate-plated: one petri dish was used for isoelectric focusing of HGPRT, one

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for analysis of glucose-6-phosphate dehydrogenase (G6PD), and a third for karyotyping (*10*).

Of the 22 hybrid clones selected on the basis of TK activity, only a single one, E7, had human G6PD indicating the presence of the human X chromosome (*11*). The karyotype of this clone included 107 to 142 chromosomes, with two mouse genomes and four to ten human chromosomes. As expected in hybrids with two mouse genomes, the G6PD isoenzyme pattern of this clone had a predominant mouse isoenzyme, a small amount of human isoenzyme, and a single heteropolymer with intermediate staining intensity (Fig. 1).

The HGPRT pattern from this hybrid was similar to that observed for G6PD. In addition to the parental isoenzymes, there was a single intermediate migrating band, the heteropolymer (Fig. 2, channels 3 and 6). The relative proportions of HGPRT activity in the mouse, heteropolymer, and human isoenzymes were 64, 29, and 8 percent, respectively (Fig. 3d). In contrast, hybrid clones lacking the human X chromosome had neither human nor heteropolymeric form of HGPRT (Fig. 2, channel 7). Another hybrid clone, derived previously from a fusion of the Cl-1D mouse cell line with HGPRT deficient human fibroblasts, had the human G6PD enzyme but neither human HGPRT nor heteropolymer (Fig. 2, channel 5). Heteropolymers were not observed when parental cells were mixed prior to being lysed (Fig. 2, channel 4; Fig. 3c). Therefore, the heteropolymer is dependent on the presence of the human X chromosome and normal human HGPRT gene.

Although pseudoisoenzymes have been eliminated, there are two isoenzyme species of human HGPRT in the fibroblast parent cells; the major isoenzyme at 7 cm is the one common to all human cells we have analyzed, and is the only band found in lymphoid cells. The minor enzyme species at 8.5 cm is unique to fibroblasts and probably re-

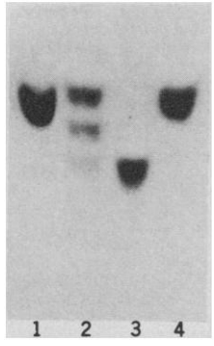

Fig. 1 (left). Cellulose acetate gel electrophoresis (*15*) of human, mouse, and hybrid G6PD. Channels: 1, mouse A_9; 2, mouse-human hybrid clone E7 with human X chromosome; 3, human fibroblast parent; and 4, mouse-human hybrid clone E5 lacking human X chromosome.

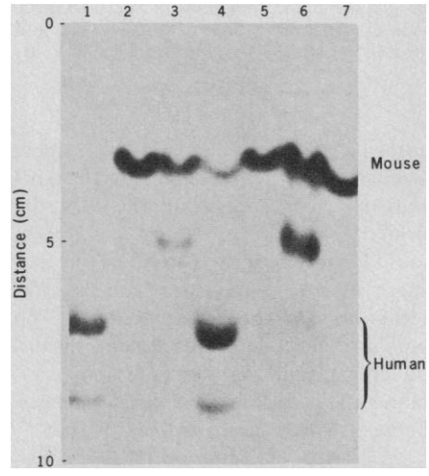

Fig. 2 (middle). Isoelectric focusing of human, mouse, and hybrid HGPRT. Trypsinized cell suspensions were rinsed with Dulbecco's phosphate-buffered saline and the cell pellets were suspended at 5×10^7 cells per milliliter in 0.01 *M* potassium phosphate, *p*H 6.8, containing 0.25*M* sucrose and 0.01*M* dithiothreitol (DTT). The cells were lysed by four cycles of freezing and thawing and centrifuged at 40,000*g* for 30 minutes at 4°C. Five microliters of the supernatants were added to 50 μl of 0.01*M* phosphate, *p*H 6.8, 0.25*M* NaCl, 0.01*M* DTT, 0.1 percent Triton X-100 in 15 percent sucrose, and incubated at 37°C for 2 hours. These samples were electrofocused in an acrylamide slab gel (0.15 by 20 cm) containing 7.5 percent acrylamide, 0.2 percent bisacrylamide, 5 percent glycerol, 2 percent ampholyte (LKB) *p*H 5 to 7, and riboflavin-5-phosphate (4 μg/ml) which had been polymerized overnight by fluorescent light. Electrofocusing was at 4°C for 20 hours at a potential of 20 V cm−1. The HGPRT activity was assayed in situ by incubating the gel at 37°C in 80 ml of a solution containing 10 m*M* phospate, *p*H 7.9, 5 m*M* $MgCl_2$, 1 m*M* DTT, 1 m*M* 5-phosphorylribose-1-pyrophosphate, and 5 μ*M* [8-^{14}C]hypoxanthine (10 μCi/μmole). After 30 minutes, the substrate solution was decanted and the gel rinsed twice with 500 ml of H_2O. The product, inosine-5-monophosphate (IMP), was precipiated in place by immersing the gel in $La(NO)_3$, as described by Bakay and Nyhan (*16*). The radioactive substrate hypoxanthine was removed by rinsing the gel overnight at 4°C with several changes of water. The gel was then dried (*17*) and autoradiographed. The *p*H gradient over the center of the gel was determined with a flat bulb *p*H electrode (Beckman 39507). The mouse band (3.3 cm) and the main human band (7 cm) were focused at *p*H 6.6 and *p*H 6.25, respectively. This major human band is isoelectrically indistinguishable from the single enzyme in human lymphoid cells, whose absolute isoelectric *p*H under these conditions is estimated to be 6.30 ± 0.10 *p*H unit (*18*). The 0- to 10-cm segment of the gel is shown. Densitometer tracings of this autoradiogram are shown in Fig. 3. Samples: 1, human; 2, mouse; 3, human-mouse hybrid E7, with human X chromosome, the same sample as in 6 but only 2 μl of the extract was analyzed; 4, mixture of human and mouse cells (mixed in ratio of three human to one mouse cell prior to lysis); 5, human-mouse hybrid, with human X chromosome, but the human X chromosome is HGPRT deficient, of Lesch-Nyhan origin (*1*); 6, human-mouse hybrid E7, with human X chromosome; and 7, human-mouse hybrid E5, lacking human X chromosome.

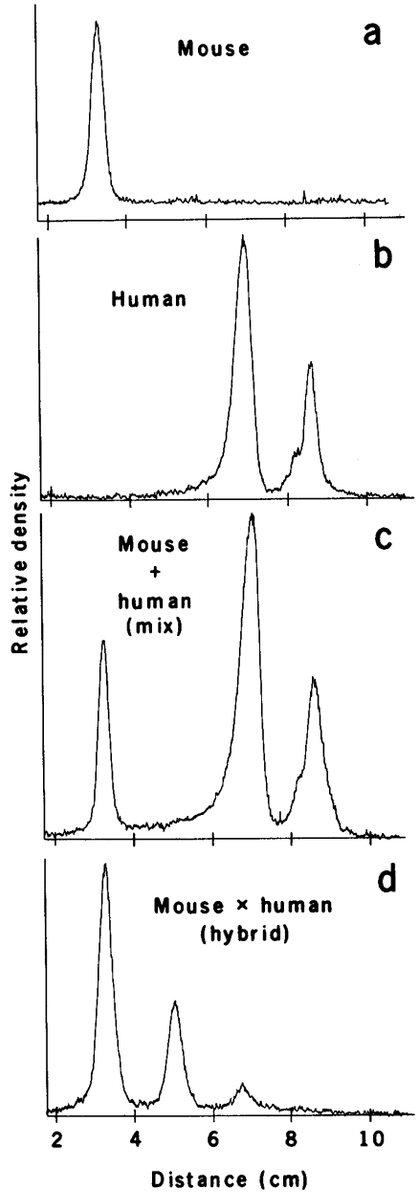

Fig. 3 (right). Distribution of HGPRT activity in isoelectric gel. Densitometer tracings of the autoradiogram shown in Fig. 2. The sample numbers of Fig. 2 are indicated in parentheses: (a) mouse (2); (b) human (1); (c) mixture of mouse and human cells (4); and (d) human-mouse hybrid E7, with human X chromosome (3). The relative quantities of HGPRT activity in the mouse, heteropolymer, and human isoenzymes were estimated by peak height analysis of densitometer scans. The percentages of activity in these species did not change substantially when this hybrid cell lysate was analyzed on another gel, nor when the concentration of the lysate was varied by a factor of 2.5, comparing channels 3 and 6 of Fig. 2.

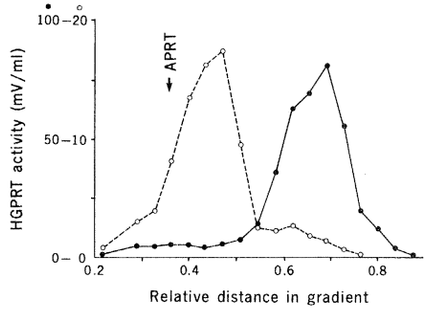

Fig. 4. Sedimentation of HGPRT of human lymphoblasts. Human lymphoblasts (PGLC 33H) (*19*), 10^7 cells per milliliter in 10 m*M* potassium phosphate, *p*H 6.8, 0.25*M* sucrose and 10 m*M* DTT, were lysed four times by freezing and thawing and then sedimented at 100,000*g* for 60 minutes. The supernatant was dialysed overnight at 4°C against the lysis buffer. Samples, containing approximately 70 μg of protein, 0.10 unit of HGPRT, and 0.03 unit of APRT (adenine phosphoribosyltransferase, E.C. 2.4.2.7) in 0.01 ml, were diluted to 0.1 ml in 2 percent ampholyte, *p*H 5 to 7, with 1 m*M* DTT (solvent A) or 1 m*M* potassium phosphate, *p*H 6.8, with 0.25*M* NaCl and 1 m*M* DTT (solvent B). The samples were centrifuged through linear 5 to 20 percent sucrose gradients (5.2 ml) in their respective solvents. Centrifugation was in the SW 50.1 rotor (Beckman) at 50,000 rev/min for 18.5 hours at 2°C. Fractions were collected from the gradient bottom by needle puncture and assayed for HGPRT and APRT. One unit of activity catalyses the conversion of 1 μmole of hypoxanthine (HGPRT) or adenine (APRT) to nucleotide per hour at *p*H 10. One unit of HGPRT activity corresponds to approximately 1 μg of HGPRT protein (*18*). Recoveries of the applied HGPRT and APRT activities in the gradients were 0.016 and 0.006 unit (solvent A) and 0.07 and 0.005 unit (solvent B), respectively. The arrow marks the sedimentation distance of the activity peak observed for the APRT enzyme, which was the same for the two gradients. The molecular weight estimates of HGPRT are based on the relation of sedimentation distance to molecular weight (*20*), and assume a molecular weight of 34,000 for the APRT (*21*). Symbols: ○, HGPRT activity in solvent A; ●, HGPRT activity in solvent B.

sults from modification of the major isoenzyme. On the other hand, the minor enzyme is not detected in the hybrid cells we have analyzed, nor in interspecific hybrids lacking mouse HGPRT (*12*). In the latter hybrids, all of the HGPRT activity is in the major band (data not shown). Therefore, the minor band does not complicate our analysis of heteropolymers in these hybrids.

Because the single HGPRT heteropolymer in hybrids indicates that these enzymes are dimers under the conditions of isoelectric focusing (relatively low ionic strength), and because previous estimates of the molecular weights of the native mouse and human enzymes [68,000 to 100,000 with a subunit size of 26,000 (*5*, *7*)] are substantially greater than that expected for dimers, we have explored the effect of the solvent on the subunit structure of human and mouse HGPRT. In Fig. 4 we compare the results obtained when HGPRT from human lymphoblasts is sedimented in the solvent of isoelectric focusing (2 percent ampholyte) with the results obtained after sedimentation at high ionic strength (0.25*M* NaCl). The sedimentation velocity of the enzyme changes substantially in these two solvents; the molecular weight of HGPRT is estimated to be 48,000 in the ampholyte solvent and 98,000 at high ionic strength, as expected for dimers and tetramers (*13*). These results indicate that, as the ionic strength of the solvent increases, dimers are converted to tetramers (*14*).

Our results demonstrate that human and mouse HGPRT subunits interact to form a single active heteropolymer. The molecular weights obtained for the enzymes sedimented at low ionic strength—the conditions of heteropolymer separation—confirm that the primary structure of the enzyme is a dimer. Furthermore, we have shown that these dimers readily associate to form tetramers at high ionic strength. The equilibrium between these two forms undoubtedly explains why previous estimates of molecular weight have ranged between dimers and tetramers (*4*, *6*).

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12. Hybrids derived from the mouse cell line A_9, which lacks HGPRT, and normal human fibroblasts.
13. The slight asymmetry in the activity peak of the enzyme at high ionic strength indicates that a small fraction of HGPRT sediments at a slower rate than that expected for a tetramer. The slower sedimenting species are not observed when experimental conditions more favorable to tetramer formation are used (G. G. Johnson, unpublished data).
14. We have observed dimeric forms of the human and mouse enzymes at low ionic strength (10 m*M* potassium phosphate, *p*H 6.8) in the absence of ampholyte; therefore, it is likely that the changes in the sedimentation velocity we observed are related to ionic strength, rather than to the presence of the ampholyte or the differences in the *p*H's of the two gradients in Fig. 4.
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Chemotactic Responses of Tumor Cells to Products of Resorbing Bone

Abstract. *To explore possible mechanisms for the metastasis of malignant cells to bone, a model of tumor cell migration was developed, using Walker carcinosarcoma or malignant lymphoma cells. It was found that bone contains a factor that is strongly chemotactic for tumor cells. This factor is released by a variety of agents that induce resorption of bone.*

Bone is one of the most common sites of metastasis of tumor cells. We have developed a model for studying the migration of tumor cells toward bone, using a type of tumor cell that frequently metastasizes to bone. We found that during the process of resorption, bone releases a factor that is strongly chemotactic for the tumor cells.

The Walker carcinosarcoma is a spontaneous rat mammary tumor that causes hypercalcemia and osteolytic bone le-

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