An Enzyme Trafficking Defect in Two Patients with Primary Hyperoxaluria Type 1: Peroxisomal Alanine/Glyoxylate Aminotransferase Rerouted to Mitochondria

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Abstract. Most patients with the autosomal recessive disease primary hyperoxaluria type 1 (PH1) have a complete deficiency of alanine/glyoxylate aminotransferase (AGT) enzyme activity and immunoreactive protein. However a few possess significant residual activity and protein. In normal human liver, AGT is entirely peroxisomal, whereas it is entirely mitochondrial in carnivores, and both peroxisomal and mitochondrial in rodents. Using the techniques of isopycnic sucrose and Percoll density gradient centrifugation and quantitative protein A–gold immunoelectron microscopy, we have found that in two PH1 patients, possessing 9 and 27% residual AGT activity, both the enzyme activity and immunoreactive protein were largely mitochondrial and not peroxisomal. In addition, these individuals were more severely affected than expected from the levels of their residual AGT activity. In these patients, the PH1 appears to be due, at least in part, to a unique trafficking defect, in which peroxisomal AGT is diverted to the mitochondria. To our knowledge, this is the first example of a genetic disease caused by such interorganellar rerouting.

PRIMARY hyperoxaluria type 1 (PH1)[1] is an autosomal recessive disease caused by a deficiency of the hepatic peroxisomal enzyme alanine/glyoxylate aminotransferase (AGT) (Danpure and Jennings, 1986). Most PH1 patients have a complete deficiency of AGT enzyme activity (Danpure and Jennings, 1988) and AGT immunoreactive protein (Wise et al., 1987). However a minority possess significant quantities of residual enzyme activity and protein. In these cases, there appears to be a relationship between the amount of residual AGT activity and various markers of disease severity (Danpure et al., 1987).

Unlike the rat, where hepatic AGT is partly peroxisomal and partly mitochondrial (Noguchi et al., 1979), and the cat, where it is entirely mitochondrial (Okuno et al., 1979), human hepatic AGT is entirely peroxisomal, both in normals (Noguchi and Takada, 1979; Yokota et al., 1987; Cooper et al., 1988*a*,*b*) and in those PH1 patients, studied so far, who possess AGT protein (Wise et al., 1987; Cooper et al., 1988*a*,*b*).

In the present study, we have investigated the intracellular distribution of AGT enzyme activity and immunoreactive protein in two PH1 patients who possessed significant amounts of residual AGT activity. It appears that these patients are examples of a unique type of trafficking defect in which peroxisomal AGT is rerouted to the mitochondria.

1. *Abbreviations used in this paper*: AGT, alanine/glyoxylate aminotransferase; PH1, primary hyperoxaluria type 1; SPT, serine/pyruvate aminotransferase.

Materials and Methods

Patients/Livers

Patient 1 was a 16-yr-old male with pyridoxine-resistant PH1. He had hyperoxaluria and hyperglycolic aciduria, a history of calcium oxalate kidney stones, and had reached end-stage renal failure requiring him to be on haemodialysis. Complete hepatectomy was performed before combined liver and kidney transplantation (see Watts et al., 1987). Patient 2 was a 33-yr-old male with pyridoxine-responsive PH1. He had a history of calcium oxalate kidney stones, and when withdrawn from pyridoxine had marked hyperoxaluria and hyperglycolic aciduria. A liver sample was obtained for verification of the diagnosis by percutaneous needle biopsy. Diagnostic percutaneous needle biopsies were also taken from controls 1 and 2 for suspected liver disease. Control 1 was a male patient suffering from Gilbert's syndrome. The liver was histologically normal. Control 2 was a female schizophrenic patient suffering from chlorpromazine toxicity. She had peroxisomal and mitochondrial proliferation and had been shown previously to have elevated levels of hepatic AGT. These two controls were chosen because preliminary immunocytochemical experiments had shown that their levels of immunoreactive AGT protein covered the extremes of the normal range (control 1 was the lowest and control 2 was the highest). Control 3 was an asymptomatic obligate PH1 heterozygote. Her hepatic AGT activity was about one-third of the normal level (Danpure and Jennings, 1988). An open liver biopsy was obtained from this individual while under general anaesthesia. Where appropriate, informed consent was given. These studies were approved by the Ethical Committee of the Harrow Area Health Authority.

For the determination of the level of residual AGT activity, samples of liver were sonicated in 0.1 mol/liter potassium phosphate buffer, pH 7.4, containing 0.1 mmol/liter pyridoxal phosphate, as described previously (Danpure et al., 1987).

Subcellular Fractionation

Sucrose Gradients. The homogenization and isopycnic sucrose gradient centrifugation were carried out as described previously (Danpure et al., 1986). Briefly, fresh samples of liver (from patient 1 only) were homogenized in 0.25 mol/liter sucrose, containing 1 mmol/liter EDTA, pH 7.4, to give a concentration of 20% (wt/vol). After centrifuging for 10 min at 600 *g*, the supernatant (4 ml) was layered onto a sucrose gradient (30 ml, 1.05–1.30 g/cm^3) and centrifuged at 72,000 *g* for 60 min in a vertical pocket rotor. Fractions (2 ml) were collected from the bottom and assayed for enzyme activity and immunoreactive AGT protein.

Percoll Gradients. An organellar fraction (from patient 1 only) was prepared by centrifuging a postnuclear supernatant at 105,000 *g* for 30 min. The pellet was gently rehomogenized in 0.25 mol/liter sucrose containing 1.0 mmol/liter EDTA, pH 7.4. An aliquot (2 ml) was mixed with 27 ml 50% Percoll in 0.25 mol/liter sucrose containing 1 mmol/liter EDTA, pH 7.4, and then layered over 1 ml 2.4 mol/liter sucrose and spun at 83,000 *g* for 45 min in a vertical pocket rotor (Neat et al., 1980). Fractions (2 ml) were collected from the bottom and assayed for enzyme activity.

Enzyme Assays

Alanine/2-oxoglutarate aminotransferase (EC 2.6.1.2), glutamate/glyoxylate aminotransferase (EC 2.6.1.4), catalase (EC 1.11.1.6), D-amino acid oxidase (EC 1.4.3.3.), cytochrome oxidase (EC 1.9.3.1), and lactate dehydrogenase (EC 1.1.2.3) were assayed as previously described (Danpure and Jennings, 1988). AGT (EC 2.6.1.44) in the sucrose and Percoll gradient fractions was assayed by the radiochemical micromethod of Allsop et al. (1987), while that in the liver sonicates was assayed using the spectrophotometric method described in Danpure and Jennings (1988). Protein was measured by the method of Lowry et al. (1951).

Immunoblotting

Samples of liver sonicates (50 μg protein) and fractions from the sucrose gradients (20 μl) were fractionated on SDS-PAGE and immunoblotted, as described previously (Wise et al., 1987), except that the first antibody (rabbit anti-human liver AGT IgG) was affinity purified by passing down an AGT-Sepharose column, absorbing against a human liver sonicate deficient in immunoreactive AGT protein, and passing down a protein A-Sepharose column. This IgG preparation detected a single 40-kD band in normal liver sonicate, identical to that produced by purified AGT. In some individuals an extra faint band at 43 kD was also detected.

Immunocytochemistry/Morphometry

Fresh liver samples were fixed in 1% glutaraldehyde and embedded in Lowicryl K4M (Carlemalm et al., 1982). Immunoreactive AGT protein was visualized by postembedding immunocytochemistry using the protein A-gold technique (Roth, 1982) as described previously (Cooper et al., 1988*b*). Catalase was visualized in the same manner, except that the antibody was sheep anti-bovine catalase (Serotec, Kidlington, Oxford, UK) diluted 1:20 and protein A-gold was replaced by protein G-gold (Bendayan, 1987). Morphometric quantitation of the gold labeling was carried out as described before Cooper et al. (1988*b*). Briefly, this involved calculating the mitochondrial and peroxisomal profile areas from randomly selected cytoplasmic fields, using a Reichert MOP Videoplan image analyzer (Kontron Instruments, Slough, Bucks, UK). Labeling density was estimated by counting the number of gold particles over each organelle or area of cytoplasm of interest and expressing this as particles per micrometers squared. All mitochondrial or peroxisomal profiles lacking gold were counted as zero values to obtain a true estimate of the mean labeling density for AGT. Such zero values were only observed in patients 1 and 2. In control livers >99% of the peroxisomal profiles showed significant label. The number of peroxisomes or mitochondria in a unit area of cytoplasm (i.e., excluding nuclei and sinusoidal spaces) was calculated from randomly chosen low-power negatives (6,850×). For each individual liver, between 1,300 and 2,000 μm^2 of cytoplasm was used to estimate the organelle frequency. We were able to estimate the "total" (T) mitochondrial or peroxisomal labeling for AGT in an average of 100 μm^2 of liver cytoplasm for each individual by the following equation:

$$T = L \times A \times N,$$

where L = the mean mitochondrial or peroxisomal gold labeling density (particles/μm^2); A = the mean mitochondrial or peroxisomal profile area (μm^2); and N = the mean number of mitochondria or peroxisomes (profiles/100 μm^2 of cytoplasm). This allows for comparison of the relative contributions made by each organelle to the total liver AGT in each patient.

Results

Both patients 1 and 2 were more severely affected than would be expected from the amounts of residual AGT activity found in their livers. Patient 1, who had 8.7% residual corrected AGT activity (Table I), had pyridoxine-resistant PH1 of such severity that he required a combined liver/kidney transplant. His clinical condition was indistinguishable from the majority of PH1 patients who had no (<2%) residual hepatic AGT activity. In comparison, the clinical condition of two other patients with similar residual AGT levels (13 and 14%) was considerably milder than that of patient 1.

Patient 2 had a milder form of pyridoxine-responsive PH1. His residual level of hepatic AGT (27.1% when corrected) was much higher than that in patient 1 (Table I). This level was similar to that found previously in asymptomatic obligate heterozygotes (Danpure and Jennings, 1988).

Isopycnic sucrose gradient centrifugation showed that total (uncorrected) AGT activity in patient 1 was partly mitochondrial and partly cytosolic (Fig. 1). Most, if not all, of the cytosolic AGT activity could be accounted for by cross-over from another enzyme glutamate/glyoxylate aminotransferase (Danpure and Jennings, 1988; Cooper et al., 1988*b*). When corrected for this cross-over (Fig. 2 *A*), the distribution of AGT activity was entirely mitochondrial. When a similar correction was applied to sucrose gradients from four other PH1 livers, any slight indication of mitochondrial activity completely disappeared (Fig. 2 *B*). Immunoblotting of the

Table I. Hepatic AGT Activities

	Enzyme Activity			
	AGT	GGT	AGT*	AGT*
				% of control
Patient 1	0.98	0.90	0.39	9
Patient 2	1.97	1.14	1.22	27
Other PH1 patients				
Mean	0.63	0.78	0.15	3
Range	(0.27–1.32)	(0.44–1.03)	(0.00–0.64)	
n	20	17	17	
Control 2	9.86	+	9.43	210
Control 3	2.00	0.84	1.45	32
Other controls				
Mean	5.00	0.65	4.50	100
Range	(3.25–8.99)	(0.38–0.92)	(2.75–8.38)	
n	16	12	12	

Units are in μmol/h per mg protein. *AGT**, AGT activity corrected for 66% cross-over from glutamate/glyoxylate aminotransferase (*GGT*). The rationale for this correction and the data for the other PH1 patients and controls are taken from Danpure and Jennings (1988). The percent values are based on the appropriate mean of other normal controls. +, the biopsy for control 2 was too small to measure GGT. Therefore the corrected AGT* value is an estimate based on the mean normal control GGT level. Patients 1 and 2, PH1 patients; control 2, schizophrenic patient with chlorpromazine toxicity; control 3, obligate PH1 heterozygote.

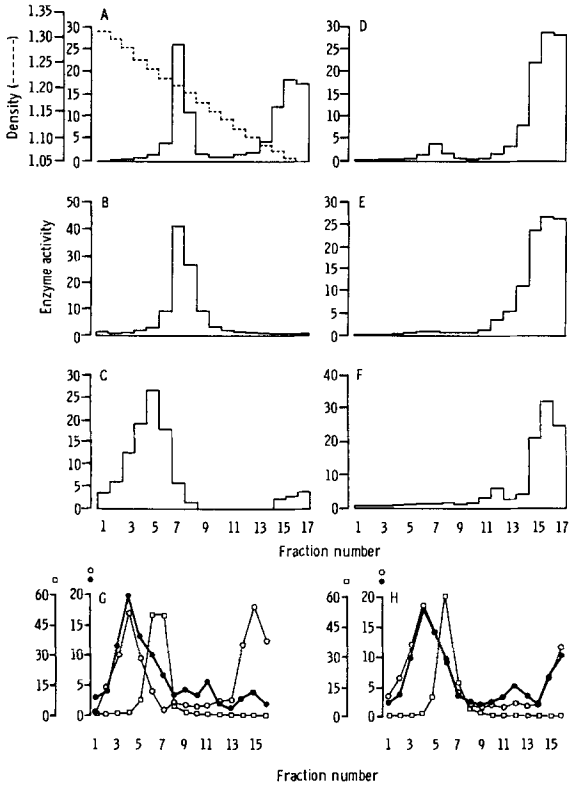

Figure 1. Subcellular distribution of total AGT enzyme activity on a sucrose gradient (patient 1). *A*, AGT; *B*, cytochrome oxidase (mitochondria); *C*, D-amino acid oxidase (peroxisomes); *D*, glutamate/glyoxylate aminotransferase (cytosol); *E*, alanine/2-oxoglutarate aminotransferase (cytosol); *F*, lactate dehydrogenase (cytosol). Fraction 1, bottom of gradient. Units of enzyme activity equal percent of total gradient activity recovered in each fraction. G and H, distribution of AGT (●), D-amino acid oxidase (○), and cytochrome oxidase (□) in normal human liver (data taken from Danpure and Jennings, 1986) and a PH1 heterozygote liver (control 3) (data taken from Danpure and Jennings, 1988).

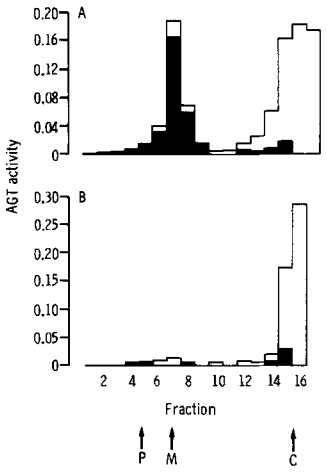

Figure 2. Subcellular distribution of "corrected" AGT activity on a sucrose gradient (patient 1). *A*, patient 1; *B*, a "typical" PH1 patient with no AGT enzyme activity (three others were almost identical). Open histogram denotes total AGT activity; solid histogram denotes AGT activity corrected for cross-over from glutamate/glyoxylate aminotransferase (Danpure and Jennings, 1988). Fraction 1, bottom of gradient. *P*, *M*, and *C*, positions of peroxisomal, mitochondrial, and cytosolic markers, respectively. Units of AGT activity equal μmol/h per mg protein.

sucrose gradient fractions showed that the 40-kD immunoreactive AGT protein was also mitochondrial (Fig. 3). An immunoreactive band of slightly higher molecular mass (~43 kD) was also found, but this was restricted to the top of the gradient (cytosolic fractions).

Confirmation of the mitochondrial location of AGT was achieved when Percoll was used. On such gradients, it could be clearly seen that the vast majority, if not all, of the AGT activity (Fig. 4) in patient 1 was associated with the mitochondria rather than the peroxisomes. Even the small amount cofractionating with the catalase (peroxisomal marker) peak might have been due to mitochondrial damage caused by resuspension of the organellar pellet, as some cytochrome oxidase, but not glutamate dehydrogenase, was also found in this fraction (Fig. 4).

Quantitative protein A–gold immunocytochemistry confirmed the results obtained by isopycnic density gradient centrifugation, both in this and previous studies (Danpure and Jennings, 1986, 1988; Cooper et al., 1988*a*,*b*). The three control livers possessed a wide range of AGT enzyme activities, which was paralleled by an equally wide range in the level of immunoreactive AGT protein, as reflected by total gold labeling (Table II, Fig. 5, *a* and *b*). As found previously (Cooper et al., 1988*a*,*b*), virtually all the gold labeling was peroxisomal, while the labeling densities in the mitochondria were hardly above the background level (Table II, Fig. 5, *a* and *b*).

Patient 1 had much lower levels of gold labeling than the controls (Table II, Fig. 5 *c*), which was compatible with his low levels of AGT enzyme activity (Table I). Nevertheless morphometric analysis confirmed that most of his immunoreactive AGT protein was mitochondrial. The gold labeling density in the peroxisomes was only ~1% of that in the controls (29–168 reduced to 0.7 particles/μm²), whereas that in the mitochondria was increased ~10 times (0.15–0.25 increased to 2.2) (Table II). It could be estimated that the total mitochondrial immunoreactive AGT protein was ~17 times greater than that contributed by the peroxisomes in patient 1 (Table II). Most of the increase in mitochondrial labeling was due to an increase in the proportion of mitochondria containing detectable immunoreactive protein (from ~4 to 42%), as the mean labeling density of only those mitochondria labeled hardly changed (Table III).

The size of the liver biopsy from patient 2 was too small to perform subcellular fractionation. However the much higher amounts of immunoreactive AGT protein in this patient made quantitative protein A–gold immunocytochemistry more conclusive than with patient 1. Patient 2 had levels of AGT enzyme activity (Table I) and immunoreactive AGT protein (represented by total gold labeling) (Table II, Fig. 5 *d*) similar to those of control 3 (an obligate PH1 heterozygote). In this patient, the mitochondrial gold labeling density was at least 20 times greater than that found in the controls (0.15–0.25 increased to 4.7 particles/μm²) (Fig. 5, Table II), whereas the peroxisomal labeling density was reduced ~20-fold (29–168 reduced to 4/μm²). However, in absolute terms, the labeling density in these two organelles were similar (4.0 compared with 4.7) (Table II). Because mitochondria are larger and more numerous than peroxisomes, the estimated total contribution of mitochondria to the hepatic immunoreactive AGT protein was ~13 times that of the peroxisomes in patient 2 (Table II). As in the case of patient 1, the main cause appeared to be the increased numbers of mito-

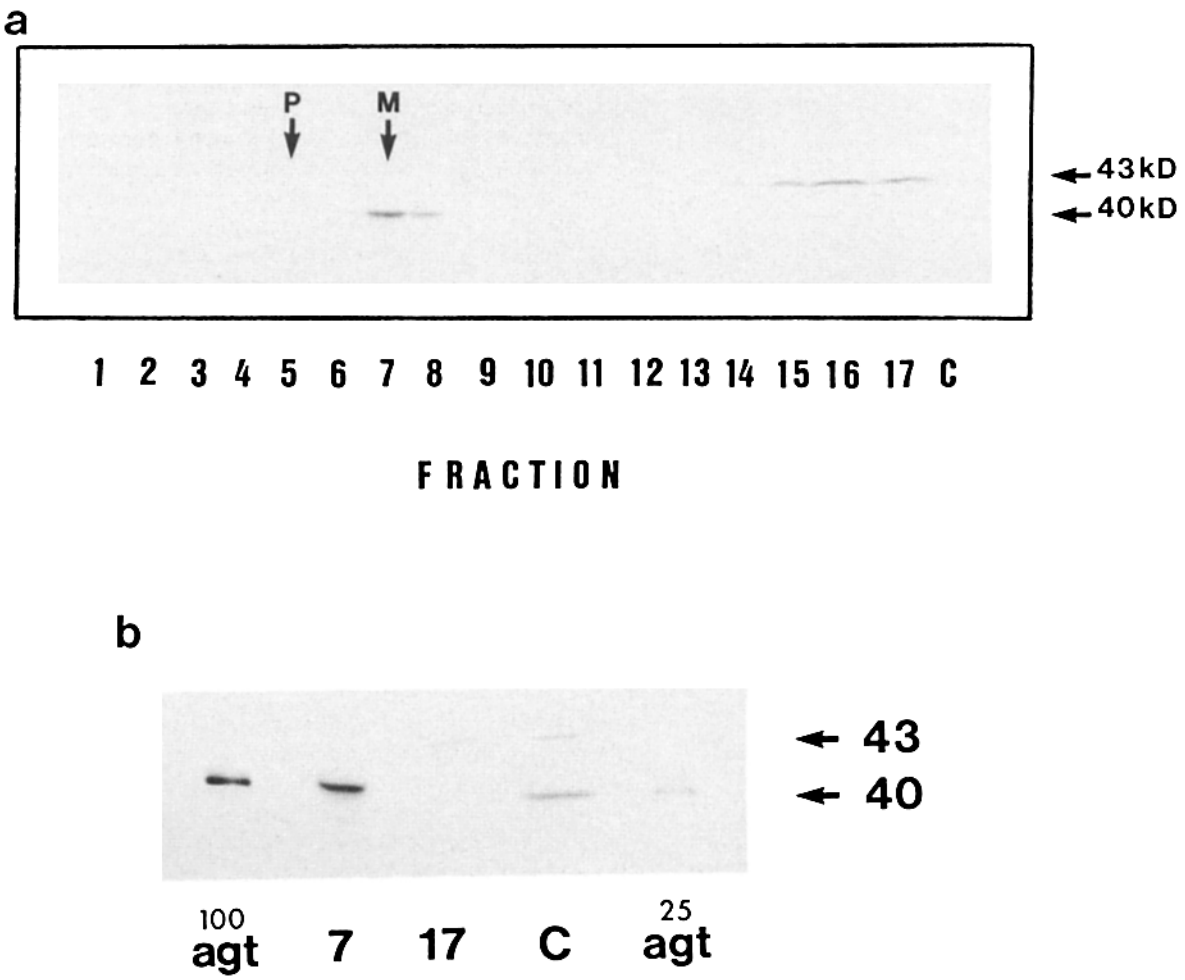

Figure 3. Immunoblot of a sucrose gradient (patient 1). (*a*) Fractions 1–17 are from the same sucrose gradient as that in Fig. 1. *C*, cytoplasmic fraction loaded onto gradient (under loaded, but showed both 40- and 43-kD bands); *P* and *M*, positions of peroxisomal and mitochondrial markers, respectively. (*b*) Selected fractions from the same gradient showing more clearly the equivalence of the mitochondrial 40-kD band and the cytosolic 43-kD band with those in the cytoplasmic (postnuclear supernatant) fraction. *7*, *17*, and *C*, fraction 7, fraction 17, and cytoplasmic fraction, respectively; *100 agt*, 100 ng AGT standard; *25 agt*, 25 ng AGT standard.

chondria labeled (Table III), but in patient 2, significant numbers of peroxisomes were also labeled (Fig 5, Table III).

This abnormal intracellular distribution of AGT appears to be specific and is not found with other peroxisomal enzymes. For example, sucrose gradient fractionation of the liver from patient 1 (Fig. 1) showed that the distribution of D-amino acid oxidase peaked at a density of ~1.24 g/cm^3, which agrees with that found before for peroxisomes in control human livers (Danpure and Jennings, 1988). In addition, when the intracellular distribution of catalase was investigated by protein A–gold immunocytochemistry in the livers of patients 1 and 2, it was found to be peroxisomal, the mitochondria being free from label (Fig. 6).

Discussion

The intracellular distribution of AGT in mammalian liver is species dependent. It is located in the peroxisomes in primates and lagomorphs (Noguchi and Takada, 1978*b*, 1979; Takada and Noguchi, 1982*b*), in the mitochondria in carnivores (Noguchi and Takada, 1978*b*; Okuno et al., 1979; Takada and Noguchi, 1982*b*) and in both organelles in rodents (Noguchi et al., 1978*a*, 1979; Noguchi and Takada, 1978*a*; Oda et al., 1982; Takada and Noguchi, 1982*b*). In human liver, its exclusive peroxisomal localization has been demonstrated using both homogenization centrifugation (Noguchi and Takada, 1979) and immunocytochemical (Yokota et al., 1987; Cooper et al., 1988*a*,*b*) techniques. To the best of our knowledge, this is the first report of mitochondrial AGT in the human.

As the severity of the disease in the two PH1 patients was considerably worse than expected simply on the basis of their overall residual AGT activity, it would appear that their particular form of PH1 was due, at least in part, to a mislocalization of their AGT. The implication of this is that, in human liver at least, AGT can not perform its metabolic function adequately, when located in the mitochondria. This is reasonable, if the major intracellular site of glyoxylate production in human liver is in the peroxisomes, as might be expected from the intracellular localization of D-amino acid oxidase (glycine → glyoxylate) and L-2-hydroxy acid oxidase (glycolate → glyoxylate) (De Duve and Baudhuin, 1966;

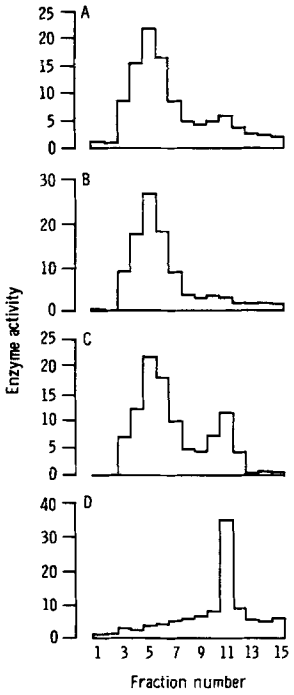

Figure 4. Subcellular distribution of AGT activity on a Percoll gradient (patient 1). *A*, AGT; *B*, glutamate dehydrogenase (mitochondria); *C*, cytochrome oxidase (mitochondria); *D*, catalase (peroxisomes). Fraction *1*, bottom of gradient. Units of enzyme activity equal percent of total gradient activity recovered in each fraction.

Peters and Seymour, 1978). A survey of the literature has failed to find any other examples of genetic diseases in mammals caused by trafficking defects leading to the rerouting or partial rerouting of an enzyme from one organelle to another (see Rosenberg et al., 1987).

The failure in the processes involved in the uptake of AGT into the peroxisomes would appear to be specific for this enzyme, as the distribution of catalase and D-amino acid oxidase was unaltered. In addition, a generalized deficiency in the uptake of peroxisomal proteins, as might occur in Zellweger's syndrome (Santos et al., 1988), would be very unlikely partly because peroxisomal morphology was relatively normal in these patients and partly because they did not suffer from any of the wide ranging symptoms characteristic of generalized peroxisomal deficiency (Schutgens et al., 1986).

Presumably the mutational event(s) directly or indirectly affect the signal peptides or organelle receptors. It is not clear whether peroxisomal and mitochondrial AGT are coded for by the same or different genes. The former is perhaps more likely because, in rats, the peroxisomal and mitochondrial forms of AGT appear to be identical (Noguchi et al., 1978*b*) as do the parts of the respective mRNAs coding for the mature enzymes (Oda et al., 1985). In addition, the identical rates of evolution of peroxisomal and mitochondrial AGT suggest that they did not arise by gene duplication (Takada and Noguchi, 1982*a*; Wilson et al., 1977).

The molecular basis for the dual location of AGT is at present unclear. Studies on serine/pyruvate aminotransferase (SPT), which is the same gene product as AGT (Noguchi and Takada, 1978*a*; Noguchi et al., 1978*b*), have shown that in rat liver, two different sized mRNAs for AGT exist (Oda et al., 1981, 1985, 1987). The larger, which codes for the mitochondrial SPT, and the mitochondrial SPT protein itself are selectively induced by gluconeogenic stimuli such as glucagon (Oda et al., 1981, 1982), the smaller mRNA and peroxisomal SPT activity remain unaltered. The cDNA for rat liver SPT has been sequenced and the putative mitochondrial signal sequence identified (Oda et al., 1987). Presumably, in humans, there has been an evolutionary loss of a functional mitochondrial signal or receptor. The molecular nature of this loss would appear to be constrained by the fact that function is restored, albeit partially, by the mutation(s) occurring in the two PH1 patients presented here.

Immunoblots of both patients' livers demonstrated the

Table II. Morphometric Analysis of Peroxisomal and Mitochondrial Gold Labeling

	Peroxisomes (P)				Mitochondria (M)					
	Gold labeling density	Perox profile area	Perox frequency	Total perox gold	Gold labeling density	Mito profile area	Mito frequency	Total mito gold	Total gold (P+M)	Ratio M/P gold
Control 1	75.0	0.21	10.1	159.0	0.15	0.27	38.3	1.6	161.0	0.01
	(2.2)	(<0.01)	(0.30)		(<0.01)	(<0.01)	(0.58)			
Control 2	168.0	0.18	19.4	587.0	0.23	0.21	65.6	3.2	590.0	0.005
	(2.9)	(<0.01)	(0.56)		(<0.01)	(<0.01)	(0.53)			
Control 3	29.0	0.18	8.2	43.0	0.25	0.26	48.3	3.1	46.0	0.07
	(1.3)	(<0.01)	(0.41)		(<0.01)	(<0.01)	(0.56)			
Patient 1	0.7	0.14	9.0	0.9	2.2	0.27	25.2	15.0	16.0	17
	(0.02)	(<0.01)	(0.42)		(0.13)	(<0.01)	(0.59)			
Patient 2	4.0	0.18	5.9	4.2	4.7	0.35	32.8	54.0	58.0	13
	(0.04)	(<0.01)	(0.35)		(0.19)	(<0.01)	(0.66)			

Control 1 is a patient suffering from Gilbert's disease. Control 2 is a schizophrenic patient suffering from chlorpromazine toxicity who showed evidence of peroxisomal and mitochondrial proliferation and high AGT activity. Control 3 is an obligate PH1 heterozygote with 30% of normal AGT activity. Patient 1 is a PH1 patient with 9% AGT activity. Patient 2 is a PH1 patient with 27% of normal AGT activity. The values are means with SEM in brackets. Gold labeling density (*L*), gold particles per micrometers squared. Profile area (*A*), organelle area in micrometers squared. Organelle frequency (*N*), number of mitochondria or peroxisomes/100 μm^2 of cytoplasm. For the estimation of mean areas and labeling densities in the peroxisomes, n = 123–203 organelles; for the mitochondria, n = 300–772. For the estimations of organelle frequency, 1,268–1,902 μm^2 of tissue were analyzed. Total mitochondrial or peroxisomal gold (*T*) is an estimate of total organellar labeling in 100 μm^2 of cytoplasm ($T = L \times A \times N$). The background (cytoplasmic) labeling varied between 0.07 and 0.19 gold particles/μm^2 (total cytoplasmic area measured varied between 560 and 1,850 μm^2 for each patient). Control incubations, using preimmune serum gave background labeling of 0.12 gold particles/μm^2 (SEM = 0.02).

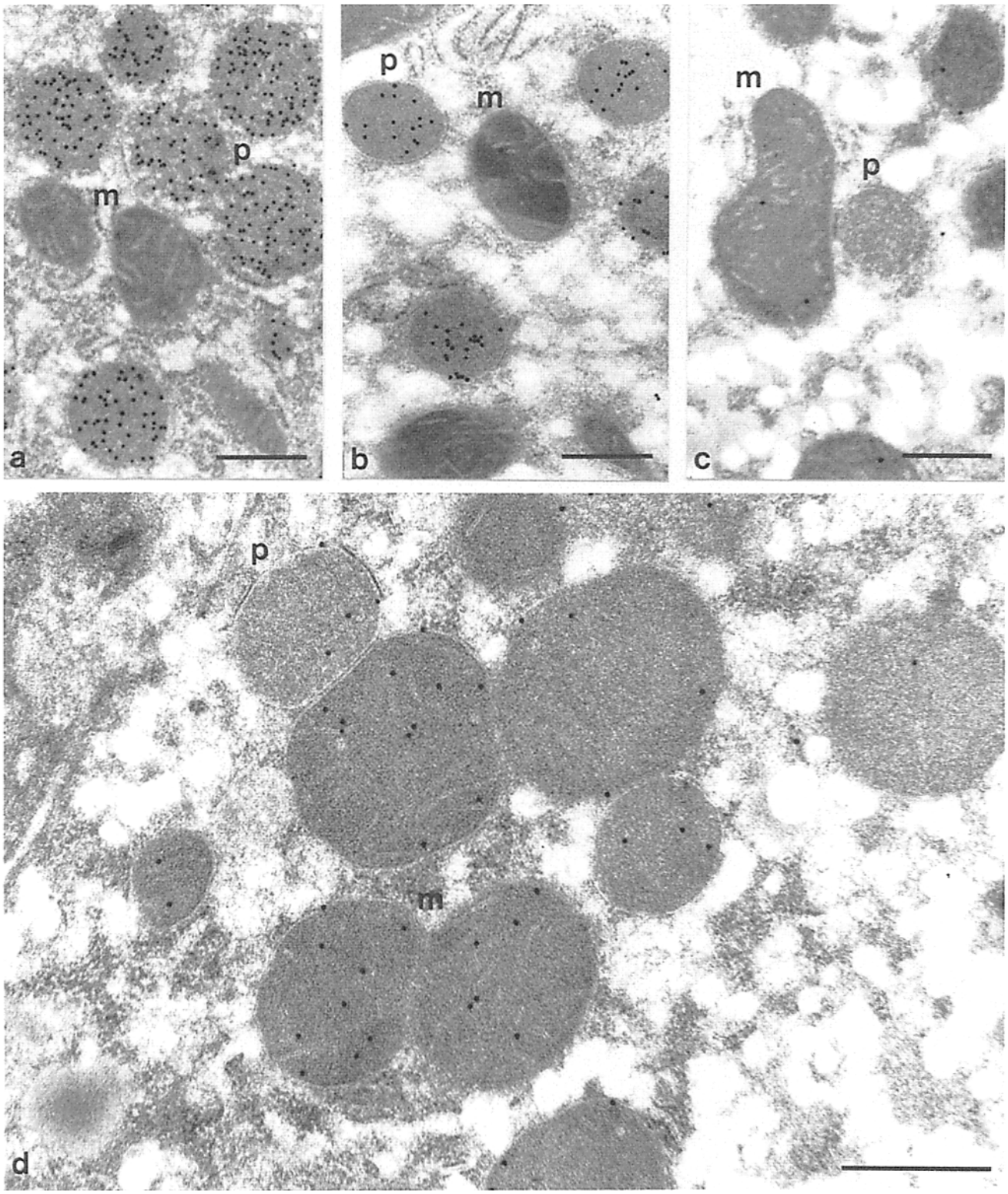

Figure 5. Protein A–gold immunocytochemistry of AGT in liver biopsies. *a*, control 2; *b*, control 3 (obligate PH1 heterozygote); *c*, patient 1; *d*, patient 2. *M*, mitochondria; *P*, peroxisomes. Bars, 0.5 μm.

presence of an immunoreactive protein with a molecular mass ~2–3 kD larger than the main AGT band. This corresponds very closely with the size of the precursor mitochondrial AGT protein in the rat, as predicted by the cDNA sequence (Oda et al., 1987). However its significance is unclear as it can also be detected in some individuals (normals and PH1 patients) who do not possess mitochondrial AGT (unpublished observations). When present, this putative precursor AGT appears to be confined to the cytosolic compartment.

Table III. Proportion of Peroxisomes and Mitochondria Labeled

	Percent of each organelle labeled		
	Peroxisomes	Mitochondria	Cytoplasm
Control 1	100 (75)	4 (4.2)	(0.07)
Control 2	100 (168)	5 (4.6)	(0.11)
Control 3	100 (29)	4 (5.8)	(0.07)
Patient 1	11 (6.3)	42 (5.3)	(0.19)
Patient 2	47 (8.4)	71 (6.6)	(0.11)

Values obtained from the morphometric analysis performed for Table II. Values in brackets equal the mean gold labeling density of the positively labeled organelle profiles only (i.e., discounting unlabeled profiles). The cytoplasmic values are equivalent to the background from Table II.

The lower amounts of AGT enzyme activity and protein in patients 1 and 2 is not surprising as they would be expected to be heterozygous for the mitochondrial AGT allele. In addition, the evolutionary inactivation of the mitochondrial route for AGT translocation in the human would have enabled random mutational events to occur, due to the relaxation of selection pressure. Reuse of this vestigial pathway results in low efficiency translocation of AGT, possibly due to alterations in posttranslational processing or receptor/signal functioning.

The molecular basis of mitochondrial signaling has received much attention but hardly anything is known with respect to peroxisomal signaling. In general, mitochondrial signal sequences are NH_2-terminal and cleaved (Schatz and Butow, 1983; Hay et al., 1984; Schatz, 1987), whereas peroxisomal signals may be COOH-terminal (Keller et al., 1987; Gould et al., 1987; Small and Lazarow, 1987) and probably not cleaved (Goldman and Blobel, 1978). The fascinating possibility exists that the AGT gene codes for, and the nascent gene product possess, both peroxisomal and mitochondrial signals. Therefore the combination of the unusual properties of AGT and PH1 may provide an extremely useful model system in which to study peroxisomal/mitochondrial targetting and the hierarchy involved (Colman and Richardson, 1986).

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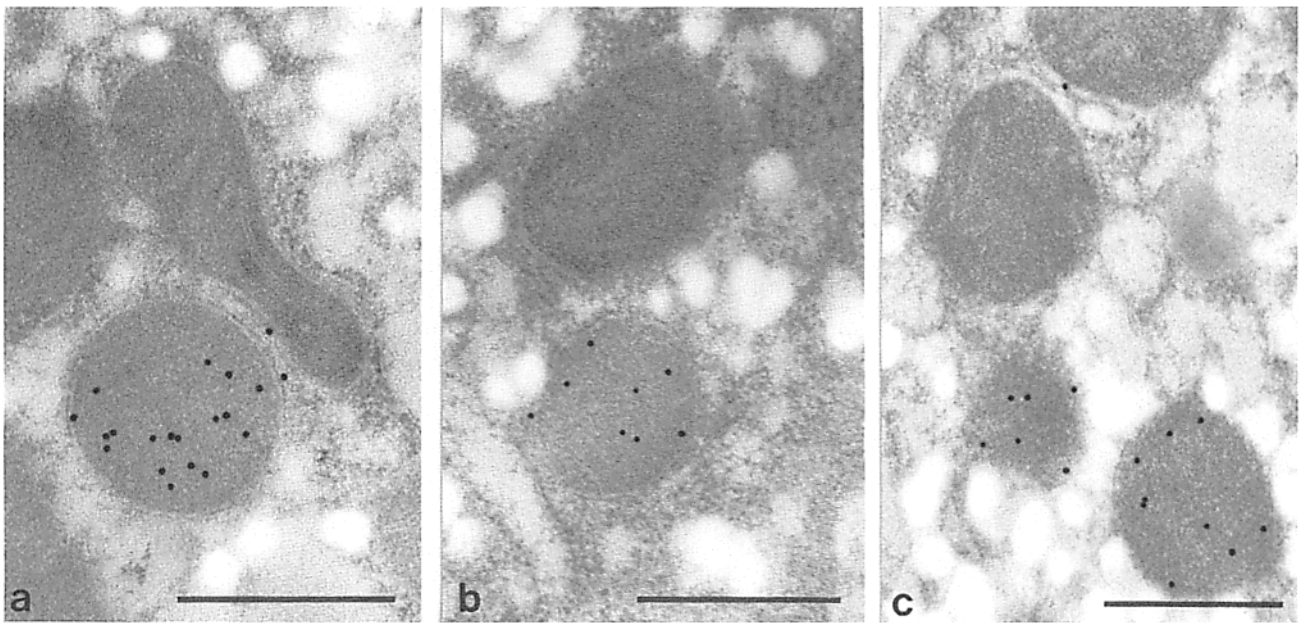

Figure 6. Protein A–gold immunocytochemistry of catalase in liver biopsies. *a*, control; *b*, patient 1; *c*, patient 2. Bars, 0.5 μm.

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