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Uroporphyrinogen Decarboxylase

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Uroporphyrinogen decarboxylase (EC 4.1.1.37) catalyzes the decarboxylation of uroporphyrinogen III to coproporphyrinogen III. The amino acid sequences, kinetic properties, and physicochemical characteristics of enzymes from different sources (mammals, yeast, bacteria) are similar, but little is known about the structure/function relationships of uroporphyrinogen decarboxylases. Halogenated and other aromatic hydrocarbons cause hepatic uroporphyria by decreasing hepatic uroporphyrinogen decarboxylase activity. Two related human porphyrias, porphyria cutanea tarda and hepatoerythropoietic porphyria, also result from deficiency of this enzyme. The roles of inherited and acquired factors, including iron, in the pathogenesis of human and experimental uroporphyrias are reviewed.

KEY WORDS: Uroporphyrinogen decarboxylase; porphyria; iron; heme biosynthesis; uroporphyrinogen; cytochrome P450IA; hepatoerythropoietic porphyria; porphyria cutanea tarda.

INTRODUCTION

Uroporphyrinogen decarboxylase (EC 4.1.1.37) (UROD) is a cytosolic enzyme that converts uroporphyrinogen III to coproporphyrinogen III (Fig. 1). Each of the four acetic acid substituents is decarboxylated in turn with the consequent formation of hepta-, hexa-, and pentacarboxylic porphyrinogens as intermediates. At high substrate concentrations, the sequence of decarboxylation is random but, under physiological conditions, decarboxylation follows a preferred, clockwise route, starting at the acetic acid substituent on ring D (Fig. 1) (Jackson *et al.*; Luo and Lim, 1993). The enzyme also decarboxylates uroporphyrinogen I and the unnatural isomers, uroporphyrinogens II and IV (Mauzerall and Granick, 1958).

Accumulation of the substrate and intermediates of the UROD reaction as porphyrinogens and, after oxidation, as porphyrins is a characteristic feature of human and experimental porphyrias in which UROD activity is decreased. Current points of interest are the mechanism of the sequential decarboxylation of uroporphyrinogen III and the pathogenesis of acquired and inherited UROD defects.

MOLECULAR GENETICS

UROD is encoded by a single gene on the short arm of chromosome 1 (1p34) in humans (Mattei *et al.*, 1985) and on a homologous region of chromosome 4 in mice (Bahary *et al.*, 1991). The gene has been cloned and sequenced from humans (Romana *et al.*, 1987), *Synechococcus sp* (*hem E*) (Kiel *et al.*, 1990), yeast (*hem 12*) (Garey *et al.*, 1992), *Bacillus subtilis* (*hem E*) (Hannson and Hederstedt, 1992), and *Escherichia coli* (*hem E*) (Nishimura *et al.*, 1993). The human gene contains 10 exons spread over 3 kb and has a 5′ promoter region organized in a way that is consistent with ubiquitous expression of a single enzyme (Romana *et al.*, 1987). Transcription is increased during erythroid differentiation (Fujita *et al.*, 1992), but the mechanism of this tissue-specific effect has not been determined.

ENZYME STRUCTURE AND MECHANISM

UROD has been purified to homogeneity from

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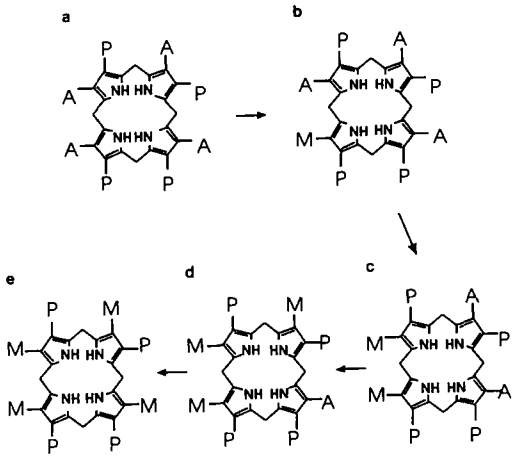

Fig. 1. The sequential reaction catalyzed by uroporphyrinogen decarboxylase. Uroporphyrinogen (a) is converted to coproporphyrinogen (e) via heptacarboxylic (b), hexacarboxylic (c), and pentacarboxylic (d) porphyrinogens. A, M, and P denote acetate, methyl, and propionate substituents, respectively.

human erythrocytes (de Verneuil *et al.*, 1983; Elder *et al.*, 1983), chicken erythrocytes (Kawanishi *et al.*, 1983), bovine liver (Straka and Kushner, 1983), yeast (Felix and Brouillet, 1990), *Rhodobacter spheroides* (Jones and Jordan, 1993), and partially purified from mouse spleen (Romeo and Levin, 1971), tobacco leaves (Chen and Miller, 1974), and *Rhodobacter palustris* (Koopman *et al.*, 1986). The mammalian, yeast, and bacterial enzymes are monomers with molecular masses of about 42 kDa; the chicken enzyme appears to be a dimer with an M_r of 72000.

Biochemical, immunochemical, and genetic studies indicate that the same monomeric protein is responsible for at least 90% of the conversion of uroporphyrinogen to coproporphyrinogen in all mammalian tissues (de Verneuil *et al.*, 1983; Elder *et al.*, 1983; Romana *et al.*, 1987; Elder *et al.*, 1989); the report by Murkerji and Pimstone (1992) of two isoenzymes in human erythrocytes has yet to be confirmed.

There are few major differences in the kinetic and physicochemical properties of the purified enzymes (de Verneuil *et al.*, 1983; Felix and Brouillet, 1990; Jones and Jordan, 1993). In contrast to most decarboxylation reactions, no coenzyme or metal requirement has been identified. At physiological pH, uroporphyrinogen III and intermediate porphyrinogens of the same isomer series are decarboxylated more rapidly by the mammalian enzyme than the corresponding series I isomers (Smith and Francis, 1981; de Verneuil *et al.*, 1983) but the rates of decarboxylation of I and III series porphyrinogens are similar at their respective pH optima (de Verneuil *et al.*, 1983).

Alignment of the predicted amino acid sequences from cloned URODs shows 59–68% similarity with the human enzyme (Fig. 2). Highly conserved regions are distributed throughout the sequence and often include one or more of the proline and glycine residues that are at the same position in all six enzymes shown in Fig. 2. These features suggest that the three-dimensional structure may also be highly conserved as might be predicted for an enzyme that catalyzes such an unusual and complex sequence of reactions. Histidine (Kawanishi *et al.*, 1983; Billi de Catabbi *et al.*, 1991; Felix and Brouillet, 1992)) and arginine residues (Billi de Catabbi *et al.*, 1991; Jones and Jordan, 1993) appear essential for activity; the latter are known to bind substrate carboxyl groups in other enymes and may do the same in UROD. These residues have not yet been identified: one histidine and two arginine residues are particularly highly conserved (Fig. 2). All URODs are inhibited by sulfhydryl group reagents, including heavy metals, and most require reducing agents for full activity *in vitro* (Romeo and Levin, 1971; Woods *et al.*, 1981; de Verneuil *et al.*, 1983; Elder *et al.*, 1983; Straka and Kushner, 1983; Felix and Brouillet, 1990; Jones and Jordan, 1993). Modification of a single cysteine residue by *N*-ethylmaleimide abolishes activity (Jones and Jordan, 1993) perhaps by producing a change in conformation that also alters immunoreactivity (Roberts, 1987). Natural mutations that specifically abolish or modify catalytic activity, and thus may be in close proximity in the folded protein, perhaps close to or within a single substrate binding cleft, have been identified in the yeast (Garey *et al.*, 1992; Chelstowska *et al.*, 1992) and human enzyme. All lie in or near highly conserved regions (Fig. 2).

The stereochemistry of decarboxylation of each of the four acetic acid substituents is identical (Barnard and Akhtar, 1979). The two methylene hydrogen atoms retain their configuration with replacement of the carboxyl group by a hydrogen atom from the solvent. A reaction mechanism, which is consistent with both this stereochemistry and the lack of requirement for a cofactor, has been proposed that involves the same group in an initial protonation at one of the α-positions of the pyrrole carrying the substituent and in the subsequent

```
                                                        D
Human  MEANGLGPQG FPELKNDTFL RAAWGEETDY TPVWCMRQAG RYLPEFRETR AAQ-DFFSTC  59
Rat    ---NGLGLQN FPELKNDTFL RAAWGEETDY TPVWCMRQAG RYLPEFRETR AAQ-DFFSTC  56
Yeast  MG-------N FPAPKNDLIL RAAKGEKVER PPCWIMRQAG RYLPEYHEVK NNR-DFFQTC  52
E.coli M--------- -TELKNDRYL RALLRQPVDV TPVWMMRQAG RYLPEYKATR AQAGDFMSLC  50
B.sub. MSKR----ET F----NETFL KAARGEKADH TPVWYMRQAG RSQPEYRKLK EKYG-LFEIT  51
Syn. . MVASS----S LP-----RLL RAARGEVLDR PPVWMMRQAG RYMKVYRDLR DKYPGFRERS  51
                            *  *          * * ***** *

          F  I                                                      S
RSPEACCELT LQPLRRFP-- LDAAIIFSDI LVVPQALGME VTMV-PSKGP SFPEPLREEQ 116
RSPEACCELT LEPVRRFP-- LDAAIIFSDI LVVPQALAME VTMV-PGKGP SFPEPLREER 113
RDAEIASEIT IQPVRRYRGL IDAAIIFSDI LVIPQAMGMR VEML-EGKGP HFPEPLRNPE 111
KNAELACEVT LQPLRRYP-- LDARSSFR-Y LTVPIAMGLG SSILKPEKVR VLPRQITCKA 107
HQPELCAYVT RLPVEQYG-- VDAAILYKDI MTPLPSIGVD VEI-KNGIGP VIDQPIRSLA 108
ETPELAIEIS LQPFRAFKP- -DGVILFSDI LTPLPGMGIP FDII-ESKGP ILEPPIRTAE 108
   *         *         *

DLEALRDPEV -VASELGYVF QAITLTRQRL AGRVPLIGFA GAPWTLMTYM VEGGGSSTMA 175
DLERLRDPAA -VASELGYVF QAITLTRQQL AGRVPLIGFA GAPWTLMTYM VEGGSFKTMA 172
DLQTVLDYKV DVLKELDWAF KAITMTRIKL DGEVPLFGFC GGPWTLMVYM TEGGGSRLFR 171
DVDKL--PIP DPEDELGYVM NAVRTIRREL KGEVPLIGFS GSPWTLATYM VEGGSSKAFT 165
DIEKLGQ--I DPEQDVPYVL ETIKLLVNE- QLNVPLIGFS GAPFTLASYM IEGGPSKNYN 165
QVAAVHDLDP EEATP--FIR PILETLRQEV GNEAAVLGFA GAPWTLAAYA IEGKSSKTYA 166
                                          **  * * **  *   **

                                                N
QAKRWLYQRP QASHQLLRIL TDALVPYLVG QVVAGAQALQ LFESHAGHLG PQLFNKFALP 235
QAKRWLYQKP VASHKLLGIL THALVPYLIG QVAAGAQALQ LFESHAGHLG SELFSKFALP 232
FAKQWINMYP ELSHKLLQKI TDVAVEFLSQ QVVAGAQILQ VFESWGGELS SVDFDEFSLP 231
VIKKMMYADP QALHALLDKL AKSVTLYLNA QIKAGAQAVM IFDTWGGVLT GRDYQQFSLY 225
KTKAFMYSMP DAWNLLMSKL ADMIIVYVKA QIEAGAKAIQ IFDSWVGALN QADYRT---- 221
NIKHLAFSEP TILHELLGKL ADNIAIYLCH QIDCGAQVVQ LFDSWAGQLS PIDYDTFALP 226
  *     *       *                   *   **      *    * *

YIRDVAKQVK ARLREAG-LA PVPMIIFAKD GHFALEELAQ AGYEVVGLDW TVAPKKAREC 294
YIRDVAKRVK AGLQKAG-LT RMPMIIFAKD GHFALEELAQ AGYEVVGLDW TVAPKKAREP 291
YLRQIAERVP KRLQELGIME QIPMIVFAKG SWYALDKLCC SGFDVVSLDW SWDPREAVKI 291
YMHKI---VD GLLRENDG-R RVPVTLFTKS ATVA-EAMAE TGCDALGLDW TTDIADARRR 280
YIKPVMNRIF SELAKEN--- -VPLIMFGVG ASHLAGDWHD LPLDVVGLDW RLGIDEARSK 277
YQQRVFQQVK AKHPE----- -VPLILYISG SAGVLERMGQ SGCDIVSVDW TVDLLDARRR 280
*                         *                          **       *

          D
VGKTVTLQGN LDPCALYASE EEIGQLVKQM LDDFGPH--R YIANLGHGLY PDMDPEHVGA 352
VGKTVTLQGE LDPCALYASE EEIGRLVQQM LNDFGPQ--R YIANLGHGLY PDMDPEHVGA 349
NKNRVTLQGN LDPGVMYGSK EVITKKVKQM IEAFGGGKSR YIVNFGHGTH PFMDPDVIKF 351
VGNKVALQGN MDPSMLYAPP ARIEEEVATI LAGFGHGEGH -VFNLGHGIH QDVPPEHAGV 339
-GITKTVQGN LDPSILLAPW EVIEQKTKEI LDQ-GMESDG FIFNLGHGVF PDVSPEVLKK 335
LGPDIGLQGN IDPGVLFGSQ DFIRDRILDT VRK--AGNQR HILNLGHGIL PGTPEDNARH 338
        **   **          *                        * ***

FVDAVHKHSR L--LRQ-N 367
FLDAVHKHSR L--LRQ-N 364
FLEECHRIGS -------K 362
FVEAVHRLSE Q--Y-H-R 353
LTAFVHEYSQ NKKMGQYS 355
FFETAKNLDQ L--LAASH 354
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Fig. 2. Alignment of predicted amino acid sequences of uroporphyrinogen decarboxylases from humans, rats, *S. cerevisiae* (yeast), *E. coli*, *B. subtilis*, and *Synechococcus* sp. strain PCC7942 (Syn). Residues identical in all six enzymes are marked by asterisks. Letters in bold above the sequence indicate six natural mutations in the yeast enzyme.

removal of the hydrogen from the O–H bond of the carboxy or in the binding of an already dissociated carboxy (Barnard and Akhtar, 1979).

There are 24 possible carboxylation sequences, involving 14 different intermediates, between uroporphyrinogen III and coproporphyrinogen III (Jackson *et al.*, 1976). UROD is capable of decarboxylating all 14 possible intermediates and yet, under physiological conditions, only one route is preferred (Fig. 1). The nature of the interactions between substrate and enzyme that determine this route has attracted much speculation but is likely to remain uncertain until the three-dimensional structure of UROD is known. The first decarboxylation of uroporphyrinogen III occurs

more rapidly than the remaining three (Tomio *et al.*, 1970), and selective inhibition of one or other of the decarboxylation steps by heating, heavy metals, changes in ionic strength, sulfhydryl-group reagents, porphyrinogens, and porphyrins has been observed for URODs from different sources (Cornford, 1964; Garcia *et al.*, 1973; de Verneuil *et al.*, 1980; Smith and Francis, 1981; Straka and Kushner, 1983; Woods *et al.*, 1984; Felix and Brouillet, 1990). These effects have mainly been interpreted as indicating the presence of two or more distinct catalytic sites. However, more recent evidence, particularly the failure to find point mutations or chemical modifications that inactivate only one part of the decarboxylation process, suggests that all decarboxylations take place within a single substrate binding pocket. Observed alterations in the pattern of accumulation of intermediates may reflect conformational changes that affect substrate binding. A model for the interaction between UROD and its substrates throughout the preferred route of decarboxylation has been proposed (Akhtar, 1994).

EXPERIMENTAL UROPORPHYRIA IN ANIMALS

Certain polyhalogenated aromatic hydrocarbons (PHAH), notably hexachlorobenzene, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and some polyhalogenated biphenyls, cause chronic hepatic uroporphyria in susceptible species and strains of mammal, including humans (De Matteis, 1991). Their administration leads, after a variable delay, to a progressive decrease in UROD activity in the liver which is accompanied by increased excretion and hepatic accumulation of uroporphyrin and other acetic acid-substituted porphyrins. Some uroporphyrin may accumulate in the liver before UROD activity falls (Visser *et al.*, 1989), but uroporphyrin is an insufficiently potent inhibitor of UROD to explain the low enzyme activities that are found, nor does its removal reactivate the enzyme (Rios de Molina *et al.*, 1980). There is immunochemical evidence that loss of activity is unaccompanied by any change in enzyme concentration, suggesting that the enzyme is inactivated by a process that targets the catalytic site without affecting major epitopes (Elder and Sheppard, 1982). More recently some nonchlorinated cyclic hydrocarbons, such as 3-methylcholanthrene and β-naphthoflavone (Francis and Smith, 1987), and the diphenyl ether herbicide formesafen (Krijt *et al.*, 1994), have been shown to cause a similar hepatic porphyria in mice.

The porphyria produced in mammals by all these chemicals is iron-dependent. Iron overload accelerates the onset of porphyria while iron depletion slows or prevents it (Sweeney, 1986; Wainstok de Calmanovici *et al.*, 1986; van Gelder *et al.*, 1993). In certain strains of mouse, long-term iron overload alone produces UROD deficiency (Smith and Francis, 1993). However, the effect of iron does not seem to be explained by direct inhibition of UROD. Acute iron overload does not alter enzyme activity. Purified human UROD is not inhibited by ferric or ferrous iron (de Verneuil *et al.*, 1983), and where *in vitro* inhibition has been demonstrated, it has required conditions that are unlikely to produce prolonged affects *in vivo* (Mukerji and Pimstone, 1986; Felix and Brouillet, 1992).

PHAHs also produce uroporphyria in chick-embryo hepatocyte cultures (Sinclair and Granick, 1974). In this system, response is rapid and uroporphyria is produced by a wider range of chemicals than in mammals (De Matteis, 1991). Uroporphyrin accumulation is less consistently accompanied by decreased UROD activity which, when it does occur, is less marked than in mammals (Lambrecht *et al.*, 1988; James and Marks, 1989). Japanese quail given hexachlorobenzene for 7–10 days also develop hepatic uroporphyria without any decrease in UROD activity (Lambrecht *et al.*, 1988), again indicating a difference between birds and mammals in response to these chemicals. Whether the underlying mechanism also differs is unclear at present; the difference may lie in the time-course of the response rather than its mechanism (Lambrecht *et al.*, 1990). Mouse hepatocyte cultures become uroporphyric after several days of exposure to chlorinated biphenyls (Sinclair *et al.*, 1990).

The mechanism of experimental uroporphyria has been investigated extensively. The great structural differences in causative chemicals, the absence of any direct evidence for the covalent binding of metabolites to UROD (Sinclair *et al.*, 1986; van Ommen *et al.*, 1989), and the iron-dependence of the uroporphyria have led to a search for a unifying hypothesis that explains all types of experimental uroporphyria (De Matteis, 1991). The recent discovery that iron alone causes uroporphyria in certain strains of mouse (Smith and Francis, 1993) provides the least complicated model yet identified. A heme precursor also appears to be required for the

inhibition of UROD, since the onset of iron-induced uroporphyria is accelerated by feeding the heme precursor, 5-aminolevulinic acid (ALA) (Smith and Francis, 1993). *In vitro* iron-dependent hydroxyl radical-generating systems oxidize uroporphyrinogen to uroporphyrin and more polar nonporphyrin products which inhibit UROD (Francis and Smith, 1988; De Matteis, 1988). Neither these inhibitors nor those isolated from rodent liver (Cantoni *et al.*, 1984; Billi *et al.*, 1986; Smith and Francis, 1987) and avian hepatocytes (James and Marks, 1989) in experimental uroporphyrias have been identified. However, the possible existence of an iron-dependent oxidative process *in vivo* leading to inhibition of UROD by an irreversible inhibitor derived from uroporphyrinogen provides an attractive, but hypothetical, mechanism for uroporphyria. The nature of the genetic factors that restrict its occurrence to certain strains of mice, and which are likely to underlie similar inherited differences in susceptibility in other species, is unknown. Although all organic chemicals, except formesafen (Krijt *et al.*, 1994), that cause uroporphyria in rodents induce microsomal cytochromes of the P450IA subfamily by a mechanism involving a receptor encoded by *Ah* locus, susceptibility does not correlate with *Ah* phenotype (Smith and Francis, 1993).

Organic chemicals that cause uroporphyria and also induce the cytochrome P450IA subfamily further accelerate the development of porphyria in mice given iron and ALA (Urquhart *et al.*, 1988). It has been suggested that these compounds may interact with the microsomal NADPH reductase-cytochrome P450 system to release stored iron from ferritin and produce reactive oxygen species (De Matteis and Stonard, 1977; Ferioli *et al.*, 1984). Hexachlorobenzene enhances iron-dependent lipid peroxidation in rodent liver (Visser *et al.*, 1989; van Gelder *et al.*, 1993), and uroporphyrin crystals and ferritin occur together in the same hepatocytes in mice given iron and hexachlorobenzene (Siersema *et al.*, 1991). Hepatic microsomes from chick embryos and rodents pretreated with 3-methylcholanthrene catalyze the NADPH-dependent oxidation of uroporphyrinogen to uroporphyrin (Sinclair *et al.*, 1987; De Matteis *et al.*, 1988; Jacobs *et al.*, 1989a), a reaction inhibited by ascorbate which also influences the development of uroporphyria in rats (Sinclair *et al.*, 1994). With avain, but not rodent, microsomes, the reaction requires a PHAH. Immunochemical and reconstitution experiments indicate that the reaction in rodents is catalyzed by cytochrome P450IA2 (Jacobs *et al.*, 1989a; Lambrecht *et al.*, 1992). Iron enhances uroporphyrinogen oxidation by microsomes (De Matteis, 1988; Bonkovsky, 1989) possibly by promoting the formation of hydroxyl radicals by NAPH-cytochrome P450 reductase (Jacobs *et al.*, 1989b) and may increase the formation of inhibitory nonporphyrin oxidation products (De Matteis, 1988).

Oxidation of uroporphyrinogen by induced chick and mouse microsomes *in vitro* is accompanied by inhibition of UROD (Lambrecht *et al.*, 1990). Although cytochrome P450-catalyzed uroporphyrinogen oxidation appears important for the production of uroporphyria in chick hepatocyte cultures, its role in intact rodent liver needs further investigation. Uroporphyria in rodents appears to occur without induction of cytochromes of the P450IA subfamily (Smith and Francis, 1993; Krijt *et al.*, 1994). However, cytochrome P450IA2 is constitutively expressed in mammalian liver, and basal rates of uroporphyrinogen oxidation may be sufficient for production of an inhibitor of UROD. Production may depend on availability of iron, particularly if iron also increases endogenous formation of ALA through induction of ALA-synthase (Bonkovsky, 1989).

UROPORPHYRINOGEN DEFICIENCY DISEASES IN HUMANS

Two human diseases, porphyria cutanea tarda (PCT) and hepatoerythropoietic porphyria (HEP), result from decreased activity of UROD in the liver. Both are characterized clinically by skin lesions caused by photosensitization by uroporphyrin and other porphyrins that accumulate as a consequence of the enzyme deficiency. Their clinical and biochemical features have been reviewed (Kappas *et al.*, 1989; Elder, 1990; Bonkovsky, 1990).

Most patients with PCT have some evidence of liver cell damage, often caused by alcohol (Kappas *et al.*, 1989) or the hepatitis C virus (Herrero *et al.*, 1993); estrogens may also precipitate symptoms (Kappas *et al.*, 1989). PCT, like experimental uroporphyria, is an iron-dependent disorder. Most patients have some degree of hepatic siderosis and about two-thirds have a small or moderate increase in total body iron stores. Iron depletion produces prolonged clinical and biochemical remission, and may return the specific activity of hepatic UROD to

normal in some patients with PCT (Elder *et al.*, 1985), while replenishment produces relapse. Iron depletion is effective therapeutically even in patients without increased liver or total body iron stores.

About 25% of patients with PCT have an autosomal dominantly inherited UROD defect which leads to half-normal UROD activity in liver and other tissues, including erythrocytes (de Verneuil *et al.*, 1978; Held *et al.*, 1989; Koszo *et al.*, 1992). The clinical penetrance of this defect is low so that only a minority of those with this form of PCT (type II or familial PCT) have a family history of the disorder. The molecular genetics of type II PCT have not yet been extensively investigated, but three different mutations have been identified which either markedly decrease the stability of UROD or produce defective pre-mRNA splicing (Garey *et al.*, 1989, 1990; Roberts *et al.*, 1995). In the other 75% of patients with PCT, UROD deficiency is restricted to the liver, enzyme activity in erythrocytes and other extrahepatic tissues being normal. The majority of these patients have no family history of PCT and are considered to have sporadic or type I PCT. A few have affected relatives (Roberts *et al.*, 1988; Held *et al.*, 1989); it is not clear whether these represent a separate category or indicate that there is an inherited predisposition to type I PCT. Although UROD activity is markedly decreased in the liver in type I PCT, the concentration of immunoreactive enzyme is normal (Elder *et al.*, 1985). Sequences of cDNAs and the promoter region of the UROD gene are normal in this type of PCT, suggesting that if any inherited factors are involved, they must be at other loci (Garey *et al.*, 1993).

The pathogenesis of PCT has been reviewed (Elder, 1990; Bonkovsky, 1990). Comparison of UROD activities in the liver in overt PCT and HEP suggests that a decrease in activity of about 80% or more is required for substrate to be overproduced to an extent that is sufficient to produce symptoms (Elder *et al.*, 1985; Roberts *et al.*, 1995). In all types of PCT, this appears to be achieved by a decrease in the specific activity of the enzyme which is caused by inhibition or inactivation of the catalytic site without any decrease in enzyme concentration. The central role of iron in the pathogenesis of PCT suggests that the mechanism of inactivation may be similar to that postulated for the experimental uroporphyrias (Elder, 1990).

Candidate loci for genetic predisposition to PCT thus include those encloding genes for iron metabolism, including the hemochromatosis gene (Adams and Powell, 1987), cytochromes of the P450IA subfamily, and hepatic ALA synthase. Hepatic concentrations of cytochrome P450IA2 show marked interindividual variation (Sesardic *et al.*, 1990) but have not yet been measured in PCT, and catalysis of uroporphyrinogen oxidation by the human cytochrome has not been reported.

Hepatoerythropoietic porphyria (HEP) is a rare cutaneous porphyria in which marked deficiency of UROD leads to the onset of skin lesions in early childhood (Kappas *et al.*, 1989; Meguro *et al.*, 1994; Roberts *et al.*, 1995). Patients are either homozygotes or compound heterozygotes for mutations of the UROD gene. Six point mutations and a deletion have been identified in patients with HEP (de Verneuil *et al.*, 1986; Romana *et al.*, 1991; de Verneuil *et al.*, 1992; Meguro *et al.*, 1994; Roberts and Elder, unpublished). All decrease catalytic activity and enzyme concentration, with the exception of one (P150A) which decreases the activity of UROD without altering its concentration (Roberts and Elder, unpublished). Only one of the mutations found in HEP homozygotes is also associated with overt PCT in heterozygotes (Roberts *et al.*, 1995). The others appear either to have a relatively mild effect on UROD activity which is not clinically manifest in heterozygotes (Megura *et al.*, 1994) or, as with the deletion reported in a compound heterozygote (de Verneuil *et al.*, 1992), have not been searched for in type II PCT.

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REFERENCES

Adams, P. C., and Powell, L. W. (1987). *Gastroenterology* **92,** 2033–2035.

Akhtar, M. (1994). In *The Biosynthesis of the Tetrapyrrole Pigments*, CIBA Foundation Symposium 180, pp. 131–152.

Bahary, N., Zorich, G., Pachter, J. E., Leibel, R. L., and Friedman, J. M. (1991). *Genomics* **11,** 33–47.

Barnard, G., and Akhtar, M. (1979). *J. Chem. Soc. Chem. Commun.* 494–496.

Billi, S. C., Wainstok de Calmanovici, R., and San Martin de Viale, L. C. (1986). In *Hexachlorobenzene* (Morris, C. R., and Cabral, J. R. P., eds.), IARC Scientific Publication No. 77. Lyon, pp. 487–491.

Billi de Catabbi, S., Rios de Molina, M. del C., and San Martin de Viale, L. C. (1991). *Int. J. Biochem.* **23,** 675–679.

Bonkovsky, H. L. (1989). *Hepatology* **10,** 354–364.
Bonkovsky, H. L. (1990). In *Hepatology* (Zakim, D., and Boyer, T. D., eds.), Saunders, Philadelphia, pp. 378–424.
Cantoni, L., Dal Fiume, D., Rizzardini, M., and Ruggieri, R. (1984). *Toxicol. Lett.* **20,** 211–217.
Chelstowska, A., Zoladek, T., Garey, J. Kushner, J., Rytka, J., and Labbe-Bois, R. (1992). *Biochem. J.* **288,** 753–757.
Chen, T. C., and Miller, G. W. (1974). *Plant Cell Physiol.* **15,** 993–1005.
Cornford, P. (1964). *Biochem. J.* **91,** 64–73.
De Matteis, F. (1988). *Mol. Pharmacol.* **33,** 463–469.
De Matteis, F. (1991). In *Hepatoxicology* (Meeks, R. G., Harrison, S. D., and Bull, R. J., eds.), CRC Press, Boston, pp. 437–479.
De Matteis, F., and Stonard, M. (1977). *Semin. Hematol.* **14,** 187–192.
De Matteis, F., Harvey, C., Reed, C., and Hempenious, R. (1988). *Biochem. J.* **250,** 13–21.
De Verneuil, H., Aitken, G., and Nordmann, Y. (1978). *Hum. Genet.* **44,** 145–151.
De Verneuil, H., Grandchamp, B., and Nordmann, Y. (1980). *Biochim. Biophys. Acta* **611,** 174–186.
De Verneuil, H., Sassa, S., and Kappas, A. (1983). *J. Biol. Chem.* **258,** 2454–2460.
De Verneuil, H., Grandchamp, B., Beaumont, C., Picat, C., and Nordmann, Y. (1986). *Science* **234,** 732–734.
De Verneuil, H., Bourgeois, F., de Rooij, F., Siersema, P. D., Wilson, J. H. P., Grandchamp, B., and Nordmann, Y. (1992). *Hum. Genet.* **89,** 548–552.
Elder, G. H. (1990). In *Recent Advances in Dermatology 8* (Champion, R. H., and Pye, R J., eds.), Churchill Livingstone, Edinburgh, pp. 55–69.
Elder, G. H., and Sheppard, D. (1982). *Biochem. Biophys. Res. Commun.* **109,** 115–120.
Elder, G. H., Tovey, J., and Sheppard, D. (1983). *Biochem. J.* **215,** 45–55.
Elder, G. H., Urquhart, A. J., De Salamanca, R. E., Munoz, J. J., and Bonkovsky, H. L. (1985). *Lancet* **1,** 229–232.
Elder, G. H., Roberts, A. G., and De Salamanca, R. (1989). *Clin. Biochem.* **22,** 163–168.
Felix, F., and Brouillet, N. (1990). *Eur. J. Biochem.* **188,** 393–403.
Ferioli, A., Harvey, C., and De Matteis, F. (1984). *Biochem. J.* **224,** 769–777.
Francis, J. E., and Smith, A. G. (1987). *Biochem. Biophys. Res. Commun.* **146,** 13–20.
Francis, J. E., and Smith, A. G. (1988). *FEBS Lett.* **233,** 311–314.
Fujita, H., Yamamoto, M., Yamagani, T., Hayashi, N., Bishop, T. R., De Verneuil, H., Yoshinaga, T., Shibihara, S., Morimoto, R., and Sassa, S. (1992). *Biochim. Biophys. Acta* **1090,** 311–316.
Garcia, R. C., San Martin de Viale, L. C., Tomio, J. M., and Grinstein, M. (1973). *Biochim. Biophys. Acta* **309,** 203–210.
Garey, J. R., Hansen, J. L., Harrison, L. M., Kennedy, J. B., and Kushner, J. P. (1989). *Blood* **73,** 892–895.
Garey, J. R., Harrison, L. M., Franklin, K. F., Metcalf, K. M., Radisky, E. S., and Kushner, J. P. (1990). *J. Clin. Invest.* **86,** 1416–1422.
Garey, J. R., Labbe-Bois, R., Chelstowska, A., Rytka, J., Harrison, L., Kushner, J. P., and Labbe, P. (1992). *Eur. J. Biochem.* **205,** 1011–1016.
Garey, J. R., Franklin, K. F., Brown, D. A., Harrison, L. M., Metcalf, K. M., and Kushner, J. P. (1993). *Gastroenterology* **105,** 165–169.
Hannson, M., and Hederstedt, L. (1992). *J. Bacteriol.* **174,** 8081–8093.
Held, J. L., Sassa, S., Kappas, A., and Harber, L. C. (1989). *J. Invest. Dermatol.* **93,** 332–334.
Herrero, C., Vicente, A., Bruguera, M., Ercilla, M.G., Barrera, J. M., Vidal, J., Teres, J., Mascaro, J. M., and Rodes, J. (1993). *Lancet* **341,** 788–790.
Jackson, A., Sancovich, H. A., Ferramola, A. M., Evans, N., Games, D. E., Matlin, S. A., Elder, G. H., and Smith, S. G. (1976). *Philos. Trans. R. Soc. London* **273,** 191–206.
Jacobs, J. M. Sinclair, P. R., Bement, W. J., Lambrecht, R. W., Sinclair, J. F., and Goldstein, J. A. (1989a). *Biochem. J.* **258,** 247–253.
Jacobs, J. M., Sinclair, P. R., Lambrecht, R. W., and Sinclair, J. F. (1989b). *FEBS Lett.* **250,** 349–352.
James, C. A., and Marks, G. S. (1989). *Can. J. Physiol. Pharmacol.* **67,** 246–249.
Jones, R. M., and Jordan, P. M. (1993). *Biochem. J.* **293,** 703–712.
Kappas, A., Sassa, S., Galbraith, R. A., and Nordmann, Y. (1989). In *The Metabolic Basis of Inherited Disease* (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D. eds.), McGraw-Hill, New York, pp. 1305–1365.
Kawanishi, S., Seki, Y., and Sano, S. (1983). *J. Biol. Chem.* **258,** 4285–4292.
Kiel, J. A. K. N., Boels, J. M., Beldman, G., and Venoma, G. (1990). *Gene* **9,** 77–84.
Koopman, G. E., Juknat de Gwerainik, A. A., and Battle, A. C. (1986). *Int. J. Biochem.* **18,** 935–944.
Koszo, F., Morvay, M., Dobozy, M., and Simon, M. (1992). *Brit. J. Dermatol.* **126,** 446–449.
Krijt, J., Vokurka, M., Sanitrak, J., Janousek, V., van Holsteijn, I., and Blaauboer, B. J. (1994). *Food Cosmet. Toxical.* **32,** 641–649.
Lambrecht, R. W., Sinclair, P. R., Bement, W. J., Sinclair, J. F., Carpenter, H. M., Buhler, D. R., Urquhart, A. J., and Elder, G. H. (1988). *Biochem. J.* **253,** 131–138.
Lambrecht, R. W., Jacobs, J. M., Sinclair, P. R., and Sinclair, J. F. (1990). *Biochem. J.* **269,** 437–441.
Lambrecht, R. W., Sinclair, P. R., Gorman, N., and Sinclair, J. F. (1992). *Arch. Biochem. Biophys.* **294,** 504–510.
Luo, J., and Lim, C.-K. (1993). *Biochem. J.* **289,** 529–532.
Mattei, M. G., Dubart, A., Beaupain, D., Goossens, M., and Mattei, J. F. (1985). *Cytogenet. Cell. Genet.* **40,** 892.
Mauzerall, D., and Granick, S. (1958). *J. Biol. Chem.* **232,** 1141.
Meguro, K., Fujita, H., Ishida, N., Akagi, R., Kurihara, T., Galbraith, R. A., Kappas, A., Zabriskie, J. B., Toback, A. C., Harber, L. C., and Sassa, S. (1994). *J. Invest. Dermatol.* **102,** 681–685.
Mukerji, S. H., and Pimstone, N. R., (1986). *Arch. Biochem. Biophys.* **244,** 619–629.
Mukerji, S. H., and Pimstone, N. R. (1992). *Int. J. Biochem.* **24,** 105–119.
Nishimura, K., Takayashiki, T., and Inokuchi, H. (1993). *Gene* **133,** 109–113.
Rios de Molina, M., Wainstok de Calmanovici, R., and San Martin De Viale, L. C., (1980). *Int. J. Biochem.* **12,** 1027–1032.
Roberts, A. G. (1987). Ph.D. thesis, University of Wales.
Roberts, A. G., Elder, G. H., Newcombe, R. G., de Salamanca, R. E., and Munoz, J. (1988). *J. Med. Genet.* **25,** 669–676.
Roberts, A. G., Elder, G. H., De Salamanca, R. E., Herrero, C., Lecha, M., and Mascaro, J. M. (1995). *J. Invest. Dermatol.*, in press.
Romana, M., Dubart, A., Beaupain, D., Chabret, C., Goossens, M., and Romeo, P. H. (1987). *Nucleic Acids Res.* **15,** 7343–7356.
Romana, M., Grandchamp, B., Dubart, A., Anselem, S., Chabret, C., Nordmann, Y., Goossens, M., and Romeo, P. H. (1991). *Eur. J. Clin. Invest.* **21,** 225–229.
Romeo, G., and Levin, Y. (1971). *Biochim. Biophys. Acta* **230,** 330–341.
Sesardic, D., Edwards, R. J., Davies, D. S., Thomas, P. E., Levin, W., and Boobis, A. R. (1990). *Biochem. Pharmacol.* **39,** 489–498.

Siersema, P. D., van Helvoirt, R. P., Ketelaars, D. A. M., Cleton, M. I., de Bruijn, W. C., Wilson, J. H. P., and van Eijk, H. G. (1991). *Hepatology* **14,** 1179–1188.

Sinclair, P. R., and Granick, S. (1974). *Biochem. Biophys. Res. Commun.* **61,** 124–133.

Sinclair, P. R., Bement, W. J., Bonkovsky, H. L., Lambrecht, R. W., Frezza, J. E., Sinclair, J. F., Urquhart, A. J., and Elder, G. H. (1986). *Biochem. J.* **237,** 63–71.

Sinclair, P. R., Lambrecht, R., and Sinclair, J. F. (1987). *Biochem. Biophys. Res. Commun.* **146,** 1324–1329.

Sinclair, P. R., Bement, W. J., Lambrecht, R. W., Gorman, N., and Sinclair, J. F. (1990). *Arch. Biochem. Biophys.* **281,** 225–232.

Sinclair, P. R., Gorman, N., Walton, H., Bement, W. J., Lambrecht, R. W., and Sinclair, J. F. (1994). *FASEB J.* **8,** A446.

Smith, A. G., and Francis, J. E. (1981). *Biochem. J.* **195,** 241–250.

Smith, A. G., and Francis, J. E. (1987). *Biochem. J.* **246,** 221–226.

Smith, A. G., and Francis, J. E. (1993). *Biochem. J.* **291,** 29–35.

Straka, J. G., and Kushner, J. P. (1983). *Biochemistry* **22,** 4664–4672.

Sweeney, G. D. (1986). *Clin. Biochem.* **19,** 3–15.

Tomio, J., Garcia, R., San Martin de Viale, L. C., and Grinstein, M. (1970). *Biochim. Biophys. Acta* **198,** 353–363.

Urquhart, A. J., Elder, G. H., Roberts, A. G., Lambrecht, R. W., Sinclair, P. R., Bement, W. J., Gorman, N., and Sinclair, J. F. (1988). *Biochem. J.* **253,** 357–362.

Van Gelder, W., Siersema, P. D., Voogd, A., Jen-Jaspars, N. C., van Eijk, H. G., Koster, J. F., de Rooij, F., and Wilson, J. H. P. (1993). *Biochem. Pharmacol.* **46,** 221–228.

Van Ommen, B., Henriks, W., Bessems, J. G. M., Gesskink, G., Muller, F., and van Bladeren, P. J. (1989). *Toxicol. Appl. Pharmacol.* **100,** 517–528.

Visser, O., van den Berg, J. W. O., Edixhoven-Bosdijk, A., Koole-Lesuis, R., Rietweld, T., and Wilson, J. H. P. (1989). *Food Cosmet. Toxicol.* **27,** 317–321.

Wainstok de Calmanovici, R., Billi, S. C., Aldonatti, C. A., and San Martin De Viale, L. C. (1986). *Biochem. Pharmacol.* **35,** 2399–2405.

Woods, J. S., Kardish, R., and Fowler, B. A. (1981). *Biochem. Biophys. Res. Commun.* **103,** 264–271.

Woods, J. S., Eaton, D. L., and Lukens, C. B. (1984). *Mol. Pharmacol.* **26,** 336–341.