The effects *in vivo* of mutationally modified uroporphyrinogen decarboxylase in different *hem12* mutants of baker's yeast (*Saccharomyces cerevisiae*)

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Nine new *hem12* haploid mutants of baker's yeast (*Saccharomyces cerevisiae*), totally or partially deficient in uroporphyrinogen decarboxylase activity, were subjected to both genetic and biochemical analysis. The mutations sites studied are situated far apart within the *HEM12* gene located on chromosome IV. Uroporphyrinogen decarboxylase activity in the cell-free extracts of the mutants was decreased by 50–100 %. This correlated well with the decrease of haem formation and the increased accumulation and excretion of porphyrins observed *in vivo*. The pattern of porphyrins (uroporphyrin and its decarboxylation products) accumulated in the cells of mutants partially deficient in uroporphyrinogen decarboxylase activity did not differ significantly, although differences *in vitro* were found in the relative activity of the mutant enzyme at the four decarboxylation steps. The excreted porphyrins comprised mainly dehydro-isocoproporphyrin or pentacarboxyporphyrin. In heterozygous *hem12-1/HEM12* diploid cells, a 50 % decrease in decarboxylase activity led to an increased accumulation of porphyrins as compared with the wild-type *HEM12/HEM12* diploid, which points to the semi-dominant character of the *hem12-1* mutation. The biochemical phenotypes of both the haploid and the heterozygous diploid resembles closely the situation encountered in porphyria cutanea tarda, the most common human form of porphyria.

INTRODUCTION

Uroporphyrinogen decarboxylase (uroporphyrinogen carboxylase, EC 4.1.1.37) is the fifth enzyme of the haem-biosynthetic pathway and catalyses sequential decarboxylation of four acetate side chains of uroporphyrinogen III to yield coproporphyrinogen III, with formation of intermediates: hepta-, hexa- and pentacarboxyporphyrinogens [1–3]. Jackson *et al.* [1] proposed that the stepwise decarboxylation of uroporphyrinogen III to coproporphyrinogen III proceeds in a clockwise fashion, starting with the acetic acid group in the D-ring of uroporphyrinogen III. This is followed by successive decarboxylation of the side groups in rings A, B and C. The acetate chains of uroporphyrinogen I are also decarboxylated to form coproporphyrinogen I, which is no further metabolized. The decarboxylation of uroporphyrinogen I proceeds randomly. All intermediate porphyrinogens with less than eight carboxy groups can serve as substrates for decarboxylase [4,5]. The enzyme has been purified from various sources [6–9], and its amino acid sequence was recently reported [10]. Despite many efforts to elucidate it, the mechanism of uroporphyrinogen decarboxylase action is still not clear [2,4–9,11–14]; in particular, there is still controversy as to whether the sequential decarboxylations are catalysed by single active centre or separate active centres are involved.

The study of uroporphyrinogen decarboxylase is of particular interest, since an inherited or acquired deficiency of the enzyme activity is the cause of the most

common human porphyria, porphyria cutanea tarda, characterized by increased excretion of uroporphyrin and some of its decarboxylation products. The molecular defects underlying these enzyme deficiencies have not yet been characterized, except in one case of homozygous porphyria cutanea tarda, where the nature of the mutation has been determined [15].

Our previous results demonstrated that the functioning of uroporphyrinogen decarboxylase in baker's yeast (*Saccharomyces cerevisiae*) is very similar to that in mammalian cells, and we described a yeast mutant with biochemical characteristics mimicking those found in porphyria cutanea tarda [16]. We isolated new mutants affected in the gene coding for uroporphyrinogen decarboxylase, namely *HEM12* [17], which could serve as new tools for investigating the mode of action of the enzyme both *in vitro* and *in vivo* [17,18].

In the present paper a comparison of the various mutants defective in *HEM12* locus was made in respect of porphyrin accumulation and haem production as a consequence of the differing extents of uroporphyrinogen decarboxylase deficiency.

EXPERIMENTAL

Strains

The principal strains of baker's yeast (*Saccharomyces cerevisiae*) used in the present study are listed in Table 1.

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Table 1. Principal strains of *S. cerevisiae* used in the present study

Abbreviations: CMG, Centre de Génétique Moléculaire, Gif-sur-Yvette, France; DGUW, Department of Genetics, University of Warsaw, Warsaw, Poland; LPBP, Laboratoire de Biochimie des Porphyrines, Université Paris VII, Paris, France.

Stock designation	Genotype	Source
SP4	α <i>leu1 arg4</i>	[31]
FL100	α <i>ura2-3</i>	[22]
IL871-1D	α <i>ura1 trp1</i>	CMG
754	α <i>leu2-3 leu2-112 his3 his4 ura3 trp1</i>	DGUW
G121	α <i>ura2-3 hem12-1 rho⁻</i>	[22]
G227	α <i>his4 hem12-7</i>	LPBP
G229	α <i>his4 hem12-8</i>	LPBP
G230	α <i>his4 hem12-9</i>	LPBP
cat9-4C	α <i>leu1 Met⁻hem12-11</i>	[17]
Sm1	α <i>leu1 arg4 hem12-2</i>	[3]
Sm2	α <i>leu2 arg4 hem12-3</i>	[17]
Sm3	α <i>leu1 arg4 hem12-4</i>	[17]
Sm6	α <i>leu1 leu2-3 leu2-112 ade2 his4-515 ctt1-1 ctt4-1 cta1-5 hem12-10</i>	[17]
Sm39	α <i>leu1 arg4 cta1-1 hem12-5 ipa1-1</i>	[17]
Sm40	α <i>leu1 arg4 cta1-1 hem12-6</i>	[17]
DSm65-2C	α <i>leu2 His-ura3 hem13-1</i>	[17]

General genetic methods

Conventional procedures of yeast genetics, namely crossing, sporulation and tetrad analysis, were used to construct strains with desired markers and to determine linkage relationships. The frequency of intragenic recombinations were estimated in two-factor crosses between *hem12* alleles by the meiotic method [19,20].

Growth conditions and preparation of cell-free extracts

Cells were grown in a complete medium containing 1% yeast extract, 1% Bacto-Peptone, 0.1% (w/v) Tween 80 (+20 mg of ergosterol/litre) and 2% (w/v) glucose (YPG-medium) or 2% ethanol (YPE-medium) as a carbon source. Cells were grown at 30 °C with vigorous aeration and harvested at the end of the exponential phase of growth, washed with water and broken with glass beads [21].

Enzyme assay

Uroporphyrinogen decarboxylase assay was performed with 2–5 mg of protein of the cell-free extracts as described in detail previously [3]. Uroporphyrinogen I or III (5–10 μ M final concn.) was used as substrate. The assay was performed anaerobically in the dark. Samples were withdrawn at determined time intervals, and porphyrins (substrates and reaction products) were analysed and quantified by h.p.l.c. The specific activity of the enzyme was estimated as the sum of the hepta-, hexa-, penta- and tetra-carboxyporphyrins (nmol) formed/h per mg of protein.

Haem content

Haem concentration in cells was determined spectrophotometrically as described previously [22].

Content of intracellular and excreted porphyrins

Concentrations of intracellular and excreted porphyrins were measured spectrophotometrically in 3 M-HCl extracts, and porphyrins were identified by the h.p.l.c. technique [3].

RESULTS AND DISCUSSION

Genetic data

Mutants, 11 in all, listed in Table 1, defective in the *HEM12* gene coding for uroporphyrinogen decarboxylase, were isolated. All of them accumulated large amounts of porphyrins and showed fluorescence under u.v. light. They were of two types: *hem12-1*, *hem12-8*, *hem12-9*, *hem12-11* could not grow on a non-fermentable carbon source because of complete haem deficiency, whereas *hem12-2*, *hem12-3*, *hem12-4*, *hem12-5*, *hem12-6* and *hem12-10*, capable of partial haem synthesis, were able to grow on a non-fermentable carbon source. The assignment of mutants for gene *HEM12* was based on enzyme-activity assay [3,22] and by means of the complementation test [17]. We established that the *HEM12* locus was linked to the *TRP1* locus (centromere-linked marker of chromosome IV) with a map distance of 35 centimorgans, and to *HEM13* locus (gene coding for coproporphyrinogen oxidase [23]) with an average map distance of 10 centimorgans. The data of tetrad analysis indicating linkage for these loci are presented in Table 2 and Fig. 1. It was found that the recombination frequencies differed depending on the *hem12* allele used in the cross with *hem13-1*. Therefore we tested the frequencies of intragenic recombination between different alleles of *hem12* mutants. Since no method is available for positive selection of wild-type recombinants (negative selection permits one to distinguish between fluorescent and non-fluorescent colonies), the recombination was studied by means of tetrad analysis. Diploids (*hem12-x/hem12-y*) were constructed, sporulated, and the tetrads were dissected and analysed for non-fluorescent wild-type recombinants. Only complete tetrads were analysed. The

Table 2. Tetrad analysis of the crosses between *HEM12*, *HEM13* and *TRP1* loci on chromosome IV

Abbreviations: PD, parental ditype tetrads; TT, tetratype tetrads; NPD, non-parental ditype tetrads; cM, map distance in centimorgans calculated from the equation:

$$cM = (TT + 6NPD)100 / (2PD + TT + NPD) \quad [19]$$

Allele pair	Number of tetrads			Map distance (cM)
	PD	TT	NPD	
<i>hem12-2</i> $\times$ <i>trp1</i>	32	57	1	35.0
<i>hem13-1</i> $\times$ <i>trp1</i>	17	23	3	47.6
<i>hem13-1</i> $\times$ <i>hem12-2</i>	66	16	0	11.1
<i>hem13-1</i> $\times$ <i>hem12-3</i>	47	17	0	13.3
<i>hem13-1</i> $\times$ <i>hem12-4</i>	48	12	0	10.0
<i>hem13-1</i> $\times$ <i>hem12-7</i>	58	10	0	7.3

Fig. 1. Map distance for *TRP1*, *HEM12* and *HEM13* loci on chromosome IV and the tentative order of *hem12* mutations

The map was constructed from the data listed in Tables 2 and 3. Mutants *hem12-4*, *hem12-9* and *hem12-11* in crosses with other *hem12* alleles sporulated and germinated very poorly, and no complete tetrads were obtained. The mutant *hem12-5* was not further analysed, since the expression of the uroporphyrinogen decarboxylase deficiency appeared to be dependent on a second mutation (T. Zoladek, unpublished work). Abbreviation: cM, centimorgan.

recombination frequencies in each cross are summarized in Table 3. They appear to be surprisingly high, but similar frequencies have been observed in yeast [24]. Although the samples of tetrads were too small for calculation of the genetic distances between the mutant sites, the values are, nevertheless, consistent and permit approximate location of the mutants within the *HEM12* locus (Fig. 1). Thus it can be unequivocally stated that the mutants studied are scattered throughout the *HEM12* gene.

Table 3. Data for two-factor crosses between *hem12* mutants

Diploids *hem12-x/hem12-y* were analysed by tetrad analysis. Spore clones which did not fluoresce under u.v. light were scored as wild-type recombinants. The recombination frequency (R.F.) was calculated as the number of wild-type recombinants/100 of spore clones.

Allele-pair cross	No. of tetrads tested	No. of wild-type spores	R.F.
<i>hem12-1</i> × <i>hem12-2</i>	159	1	0.15
<i>hem12-1</i> × <i>hem12-3</i>	69	1	0.36
<i>hem12-1</i> × <i>hem12-10</i>	80	1	0.31
<i>hem12-2</i> × <i>hem12-6</i>	102	1	0.24
<i>hem12-2</i> × <i>hem12-3</i>	103	2	0.48
<i>hem12-3</i> × <i>hem12-6</i>	92	1	0.27
<i>hem12-8</i> × <i>hem12-3</i>	102	3	0.73
<i>hem12-10</i> × <i>hem12-7</i>	91	3	0.82
<i>hem12-6</i> × <i>hem12-7</i>	92	5	1.35
<i>hem12-5</i> × <i>hem12-6</i>	50	2	1.00
<i>hem12-5</i> × <i>hem12-3</i>	53	3	1.4

Uroporphyrinogen decarboxylase activity in cell-free extracts of *hem12* mutants

The accumulation of decarboxylation products of uroporphyrinogen I and III incubated anaerobically with extracts of wild-type and mutant cells was compared at a fixed substrate concentration (10 μ M and 8 μ M respectively) and 2–5 mg of protein. For the enzyme assay, the mutants partially defective in haem synthesis were grown on glucose or on ethanol. The extracts of cells grown on either carbon source showed the same pattern of accumulation of decarboxylation products, though the activity was higher in the cells grown on ethanol. In the present paper, only the data obtained with the latter cells are presented. The totally-haem-deficient mutants had to be grown on glucose.

The data presented in Table 4 show that the enzyme activity in extracts from mutant cells was significantly decreased and corresponded to 0–30% (for uroporphyrinogen III) and 0–60% (for uroporphyrinogen I) of the values for the standard strain. The specific activity as a measure of the enzyme deficiency was expressed in a simplified manner as the sum (nmol) of hepta-, hexa-, penta-carboxy- and coproporphyrinogens formed during 1 h of the reaction. This, however, does not fully reflect the kinetics of successive decarboxylations. To illustrate the complexity of the reaction, the relative rates of accumulation of different decarboxylation products catalysed by parental and five mutant enzymes are presented graphically (Fig. 2a and 2b).

As expected, the completely-haem-deficient mutants, *hem12-7* and *hem12-8*, like the previously described mutant *hem12-1* [22], contained no detectable activity of the enzyme. In the *hem12-9* mutant the activity was barely detectable; unexpectedly, the specific activity of the enzyme in the *hem12-11* mutant was of the same magnitude as that in mutants partially blocked in haem synthesis. However, only the products of the first and second decarboxylations were found. As shown in Fig. 3, the formation of hepta- and hexa-carboxyporphyrinogen was linear over 1 h, and heptacarboxyporphyrinogen was the species mainly accumulated (85%).

As for the mutants partially defective in haem synthesis, from the graphs presented in Fig. 2(a) it appeared that the enzyme in the *hem12-10* strain was almost inactive in the 'in vitro' assay, and it was rather surprising to find that haem was synthesized in the mutant cells (Table 4, Fig. 4). It seems that mutation in this case led to a defect in the first decarboxylation step and, in consequence, formation of further reaction products was dramatically diminished.

The alternative explanation is that the mutation led to an instability of the defective enzyme *in vitro*. Also, in the *hem12-6* mutant, the diminished accumulation of the first decarboxylation product was accompanied by a marked decrease of the rates of subsequent reactions. In mutants *hem12-3* and *hem12-4* the accumulation of heptacarboxyporphyrinogen was similar to that in the parental strain, whereas the rates of accumulation of all other decarboxylation products were diminished; these differences were more pronounced in the *hem12-4* than in the *hem12-3* strain. It should be emphasized that, in all the mutants studied, formation of coproporphyrinogen was impaired to the greatest extent.

The decarboxylation of uroporphyrinogen III by the wild-type enzyme was faster than that of its isomer I

Table 4. Effects *in vivo* of modified uroporphyrinogen decarboxylase activity in the different *hem12* mutants

Measurements were performed as described previously [3] with cells harvested at the end of the exponential phase of growth. The enzyme activity was measured in acellular extracts and estimated as the sum (nmol) of hepta-, hexa-, penta- and tetra-carboxyporphyrins formed/h per mg of protein, from uroporphyrinogen III and uroporphyrinogen I. Haem and porphyrin contents were measured in whole cells. Since the values obtained did not vary by more than the accuracy of the methods ($\pm 5\%$ for separate experiments), results shown represent single assays in one experiment. Abbreviation: n.d., not detectable.

Carbon source	Strain	<i>hem12</i> allele	Activity <i>in vitro</i> of uroporphyrinogen decarboxylase (nmol of products/h per mg of protein)		Haem (nmol/g dry wt.)	Phenotype <i>in vivo</i>	
			Isomer III	Isomer I		Porphyrins (nmol/g dry wt.)	
						Intracellular	Excreted
Glucose (2 %)	SP4	<i>HEM12</i>	0.255	0.198	70	2	5
	G121	<i>hem12-1</i>	n.d.	n.d.	≤ 1	130	n.d.
	G227	<i>hem12-7</i>	n.d.	n.d.	≤ 1	86	n.d.
	G229	<i>hem12-8</i>	n.d.	n.d.	≤ 1	41	n.d.
	G230	<i>hem12-9</i>	0.008	0.007	≤ 1	21	n.d.
	cat9-4C	<i>hem12-11</i>	0.098	0.062	≤ 1	42	9
Ethanol (2 %)	SP4	<i>HEM12</i>	0.365	0.277	222	8	13
	Sm2	<i>hem12-3</i>	0.101	0.098	129	100	9
	Sm3	<i>hem12-4</i>	0.133	0.170	46	136	27
	Sm40	<i>hem12-6</i>	0.065	0.036	43	183	5
	Sm6	<i>hem12-10</i>	0.034	0.073	19	265	21

(Table 4), which is in agreement with the data reported for rat liver uroporphyrinogen decarboxylase [4]. With uroporphyrinogen III as a substrate, coproporphyrinogen accumulated at the highest rate (Fig. 2a), whereas with uroporphyrinogen I as a substrate the rate of coproporphyrinogen accumulation was significantly lower (Fig. 2b). In mutants, independently of the substrate used, almost no coproporphyrinogen was formed. Therefore, the impairment of the fourth decarboxylation step in mutants has smaller effect on the total activity with uroporphyrinogen as a substrate.

In all mutants except *hem12-11* the formation of heptacarboxyporphyrinogen I was diminished. Similarly, as with uroporphyrinogen III, the activity of the enzyme was most affected in *hem12-6* and *hem12-10* cells.

The assay of uroporphyrinogen I and III decarboxylase activity *in vitro* revealed differences in the rates of accumulation of particular decarboxylation products in *hem12* mutants. However, only in strain *hem12-11* did the mutation lead specifically to the blocking of the third decarboxylation step (Fig. 3). This is the only mutant which seems to be affected in the specific catalytic site, suggesting the existence of more than one such site. The total lack of any of the decarboxylation steps with either substrate (uroporphyrinogen III and I) in the mutants *hem12-1*, *hem12-7*, *hem12-8* and *hem12-9* could be explained in five alternative ways: (1) absence of protein (nonsense or frameshift mutations); (2) instability of the enzyme; (3) inability to bind the substrate; (4) inactivation of catalytic site (assuming that there is only one); or (5) inactivation of the catalytic site responsible for the first decarboxylation step if there is more than one active centre.

In the other mutants with a residual activity of the enzyme the different mutations, though scattered within the gene, affected all the decarboxylation steps. The data

do not favour the four-active-centre hypothesis; however, nor do they prove that there is only one such centre.

The reaction catalysed by uroporphyrinogen decarboxylase is very complex: a single enzyme catalyses four successive decarboxylation steps. The decarboxylated substrates, porphyrinogens, and porphyrins (the oxidized intermediates) act as inhibitors of the reaction [4,5,8,9]. This complexity precludes unequivocal interpretation of the presented data.

The comparison of the kinetics of decarboxylation using hepta-, hexa- or penta-carboxyporphyrinogens from isomeric series III and I and identification of the isomer type of the intermediates would have allowed more definite conclusions to have been made.

Effect *in vivo* of uroporphyrinogen decarboxylase deficiency in *hem12* mutants

As described previously [3], partial uroporphyrinogen decarboxylase deficiency in the *hem12-2* mutant led to the accumulation in cells of uroporphyrin and heptacarboxyporphyrin, accompanied by a low content of haem. It seemed worthwhile, therefore, to examine how the modification of uroporphyrinogen decarboxylation steps found *in vitro* for other *hem12* mutants influenced functioning of the defective enzyme *in vivo*.

As shown in Table 4 and Fig. 4, a large amount of porphyrin was accumulated in mutant cells, exceeding 10–40 times that found in wild-type cells. The amount of excreted porphyrins was 1–5 times higher than in the wild-type (Table 4). There was a distinct correlation between the level of uroporphyrinogen decarboxylase activity and haem and porphyrin production. The lower the enzyme activity, the less haem was formed and the greater was the accumulation of porphyrins. However, there was no overproduction of porphyrins: the production of tetrapyrroles expressed as the sum of

Fig. 2. Accumulation of decarboxylation products uroporphyrinogen III (a) and uroporphyrinogen I (b) when incubated anaerobically with cell-free extracts obtained from wild-type parent (WT) and *hem12* mutants grown on ethanol medium

The conditions of the assay were as described in [3]. Δ , $\circ$ and $\square$, hepta-, hexa- and penta-carboxyporphyrins; $\blacktriangledown$, coproporphyrin. The presence of heptacarboxyporphyrin at zero time is due to minor contamination of the substrate, uroporphyrin, with this porphyrin. The pattern of porphyrin accumulation was highly reproducible, so only the results corresponding to one experiment are shown.

intracellular and excreted porphyrin intermediates, as well as cellular haem concentration, were almost the same in parental and mutant cells. This indicates that porphyrin accumulation in *hem12* mutants results from uroporphyrinogen decarboxylase deficiency and not from impaired regulation of the haem-biosynthetic pathway.

An analysis of the nature of porphyrins accumulated

revealed that, in *hem12-7* and *hem12-8* mutants, where haem synthesis is totally blocked, uroporphyrin was practically the only accumulated product, as in the *hem12-1* strain (Fig. 5) [22]. In the *hem12-11* mutant the pattern of intermediates accumulated resembled that of *hem12-2* [3]. The appearance of penta- and tetra-carboxyporphyrins in *hem12-11* cells is surprising, since, in the assay *in vitro*, no detectable activity relating to the

Fig. 3. Accumulation of decarboxylation products from uroporphyrinogen III (UroIII) ($8 \mu\text{M}$) and I (UroI) ($10 \mu\text{M}$) when incubated anaerobically with a cell-free extract obtained from *hem12-11* mutant cells grown on glucose medium

For details and explanation of symbols, see Fig. 2.

last two decarboxylation steps was found. Probably *hem12-11* is a 'leaky' mutation, and during 18 h of growth the intermediates, which are produced at a very low rate, can accumulate. Therefore it seems that the

assay *in vivo* reflects more faithfully the functioning of the modified enzyme in the cell.

In all mutants with residual activity of uroporphyrinogen decarboxylase, including *hem12-11*, uroporphyrin was the main accumulation product. It accounted for 40–70 % of the total porphyrins. The proportion of hepta-, hexa- and penta-carboxy intermediates did not differ significantly, although in *hem12-11*, as in *hem12-2*, heptacarboxyporphyrin predominated. There was a small amount of coproporphyrin or, as in *hem12-10*, none at all. The excreted porphyrins comprised mainly pentacarboxyporphyrin and dehydroisocoproporphyrin (up to 50 % of total porphyrins excreted; data not shown), whereas wild-type cells excreted mainly coproporphyrin (74 %).

From the results presented it appears that, irrespective of the extent of the enzyme deficiency and of the differences in the functioning of the mutated enzyme *in vitro*, the proportion of accumulated and excreted porphyrins remained similar. The same has been found in mammals; a more or less severe decrease of uroporphyrinogen decarboxylase activity caused by mutation or treatment with chemicals resulted in a similar pattern of porphyrins excreted in the urine or faeces, or accumulated in liver [25,26], which suggest the same mode of enzyme action in yeast and mammals.

It is not surprising that, in porphyria cutanea tarda, the porphyrin accumulated in liver and excreted in urine consists not only of uroporphyrin [5], since the mutants accumulating only uroporphyrin were characterized by a total lack of uroporphyrinogen decarboxylase activity, leading to a lack of haem. Such a lesion would be lethal

Fig. 4. Total haem content and intracellular porphyrin accumulation in the wild-type (WT) parent and *hem12* mutant strains grown on ethanol

For each strain, the first black bar represents haem, the second hatched bar represents porphyrins and the open bars represent the percentage of each porphyrin accumulated. Key to numbers: 8, 7, 6, 5, 4+i indicate uro-, hepta-, hexa-, penta- and (copro- + dehydroiso-coproporphyrin) respectively.

Fig. 5. Intracellular porphyrin accumulation in the wild-type (WT) parent and *hem12* mutant strains totally haem-deficient grown on glucose

For details, see Fig. 4.

for any obligatory aerobe, whereas *S. cerevisiae* can survive owing to its ability to ferment.

Expression of uroporphyrinogen decarboxylase deficiency in a heterozygous diploid

We compared the profiles of porphyrins accumulated in the cells of mutants deficient in uroporphyrinogen decarboxylase activity with those found in human porphyria cutanea tarda. Such a comparison is applicable only to the heritable form of that porphyria. It is generally maintained that one form of the disease is inherited as an autosomal dominant trait [5,25–28]. To study the possible interaction between the wild-type and mutant *hem12* alleles in yeast heterozygotes, we constructed a homozygous wild-type diploid, *HEM12/HEM12* (FL100/SP4), and a heterozygous one, *hem12-1/HEM12* (G121/SP4). In *hem12-1* mutant cells, owing to the complete block of uroporphyrinogen decarboxylase activity, only uroporphyrin was accumulated [22]. According to the criteria applied to screen the *hem12* mutants, i.e. visible porphyrin accumulation in low-temperature spectra of whole cells and fluorescence under u.v. light [17,22], it was assumed that the *hem12-1* mutation was recessive. The detailed analysis of porphyrins accumulated in diploid cells revealed the difference between the wild-type and *hem12-1/HEM12* strains, indicating that *hem12-1* mutation was not entirely recessive. The results presented in Table 5 and Fig. 6 show that the level of intracellular porphyrins in the

Fig. 6. Pattern of intracellular porphyrins accumulated in homozygous *HEM12/HEM12* and heterozygous *HEM12/hem12* diploid cells grown on ethanol

The values in parentheses represent the total concentrations of porphyrins in nmol/mg dry wt. Key to numbers: 8, 7, 6, 5, uro-, hepta-, hexa- and penta-carboxyporphyrin; 4, coproporphyrin.

heterozygous diploid was about 3 times higher than in the wild-type homozygote. The elevated level of porphyrins was due mainly to accumulation of uroporphyrin, but the amount of hepta-, hexa- and penta-carboxy intermediates was also increased, similarly to single *hem12* mutants partially deficient in uroporphyrinogen decarboxylase activity. The accumulation of porphyrins in the heterozygote was correlated with a 50 % decrease in the activity of uroporphyrinogen decarboxylase (results not shown). However, no decrease in haem content was observed.

From the present results it appears that the increased concentration of uroporphyrin and its decarboxylation products in the heterozygote tested was insufficient to produce visible fluorescence of cells or visible porphyrin peaks in the spectra of whole cells. Our preliminary results suggest some resemblance of these heterozygotes to familial cases of porphyria cutanea tarda [5,26–30], where, most often, the diminished uroporphyrinogen decarboxylase activity was not clinically expressed. However, it may be assumed that this disorder of porphyrin metabolism is due to a genetic predisposition and that its expression can be induced by toxic factors or additional mutations. The preliminary data presented here point to the usefulness of studying the diploids heterozygous for *hem12* mutant alleles.

The analysis of porphyrins in number of diploids with different combinations of *hem12* alleles and the possibility of induction of new mutations changing the expression of mutant alleles in diploids provide us with a relatively simple system for studying the genetic defects underlying the differences in the expression of uroporphyrinogen decarboxylase deficiency.

Table 5. Accumulation of porphyrins by diploid strains: homozygotic *HEM12/HEM12* and heterozygotic *hem12/HEM12*

Assay conditions were as described in Table 4. Abbreviations: 8, uroporphyrin; 7, hepta-, 6, hexa- and 5, penta-carboxyporphyrin; 4, coproporphyrin.

Strain	<i>hem12</i> allele	Haem (nmol/g dry wt.)	Intracellular porphyrins (nmol/g dry wt.)					
			Total	8	7	6	5	4
FL100/SP4	<i>HEM12/HEM12</i>	250–300	4	1.5	0.9	0.8	0.1	0.7
G121/SP4	<i>hem12-1/HEM12</i>	250–300	13	7.9	1.7	2.3	0.5	0.6

We are deeply grateful to Professor Aleksandra Putrament for critical reading of the manuscript. Part of this work was carried out during the stays in Paris of A.K. and J.R., who acknowledge gratefully the fellowships as 'Boursier de la Convention d'Echanges C.N.R.S.-Polish Academy of Sciences.' This work was supported in part by the Polish Academy of Sciences (project CPBR 3.13) and in part by the Centre National de la Recherche Scientifique, the University Paris VII and the Fondation pour la Recherche Medicale.

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Received 5 October 1987/16 December 1987; accepted 10 February 1988