The alternative pathway of haem synthesis via dehydroisocoproporphyrinogen in mutants of *Saccharomyces cerevisiae* partially deficient in uroporphyrinogen decarboxylase activity

Porphyrias, disorders in haem biosynthesis in humans, result from defects of individual enzymes in the haem-biosynthetic pathway. Uroporphyrinogen decarboxylase (uroporphyrinogen carboxylase, EC 4.1.1.37), the fifth enzyme of this pathway, is of particular interest since an inherited or acquired deficiency of this enzyme causes the most common porphyria, porphyria cutanea tarda (PCT) [1–3]. The pattern of porphyrins excreted in PCT comprises uroporphyrin (the substrate of the enzyme) and hepta-, hexa-, and pentacarboxyporphyrins (intermediates with less than eight carboxy groups) together with copro- and isocoproporphyrin series. Isocoproporphyrins, excreted only in trace amounts by healthy humans, comprise up to 36% of total porphyrins excreted by patients suffering from PCT [4,5].

It has been postulated that dehydroisocoproporphyrinogen (belonging to the isocoproporphyrin group) is formed from pentacarboxyporphyrinogen by coproporphyrinogen III oxidase (EC 1.3.3.3), the next enzyme of the pathway. Usually, coproporphyrinogen oxidase does not metabolize pentacarboxyporphyrinogen but converts coproporphyrinogen into protoporphyrinogen. However, an additional metabolic route has been postulated comprising the conversion of pentacarboxyporphyrinogen to dehydroisocoproporphyrinogen, which is later converted to harderoporphyrin and then to haem (see Fig. 1) [4–7]. This second pathway seems to be active only when pentacarboxyporphyrinogen is present in cells in a significant excess, as is found in individuals suffering from PCT.

We previously demonstrated [8,9] that the functioning of uroporphyrinogen decarboxylase in baker's yeast (*Saccharomyces cerevisiae*) is very similar to that in mammalian cells. We also described several mutants with biochemical characteristics almost identical to those found in PCT [8–10]. These mutants accumulated and excreted vast amounts of intermediates of uroporphyrinogen decarboxylation, together with dehydroisocoproporphyrin.

The above mutants enabled us to verify the existence of the alternative metabolism of pentacarboxyporphyrinogen in yeast. In order to check whether pentacarboxyporphyrinogen is metabolized by coproporphyrinogen III oxidase we constructed strains bearing two mutations: one in gene HEM13 coding for coproporphyrinogen III oxidase, and another in gene HEM12 coding for uroporphyrinogen decarboxylase. The mutation in gene HEM13 (called *hem13-1*) caused lack of the activity of the coproporphyrinogen oxidase [11] whereas mutations in gene HEM12 (*hem12-2*, *hem12-3*, *hem12-4*, *hem12-10*) caused partial deficiency in uroporphyrinogen decarboxylase activity (about 13–52% of the activity detected in wild-type strains [9]). Double mutants *hem12 hem13-1* were unable to synthesize haem and hence required a fermentable carbon source for growth (glucose in our experiments, as described in [9]). In such conditions haemless mutants obtain energy from glucose fermentation. The level of coproporphyrinogen oxidase in cells grown in the presence of glucose is significantly higher than in cells grown in the absence of glucose. This results from the negative control of coproporphyrinogen oxidase synthesis by haem [11,12], and haem synthesis is, in turn, negatively controlled by glucose [13,14].

Thus, we compared the porphyrin patterns of strains bearing a mutation in gene HEM12 (decreased uroporphyrinogen decarboxylase activity accompanied by a high level of the copro-

Fig. 1. Alternative pathways for the metabolism of pentacarboxyporphyrinogen postulated by Elder [5]

The isomer of pentacarboxyporphyrinogen shown in this figure is an example since the position of the acetic acid substituent has not been established. Abbreviations: pentacarboxy-, pentacarboxyporphyrinogen; dehydroisocopro-, dehydroisocoproporphyrinogen; harderoporphyrinogen, harderoporphyrinogen; proto-, protoporphyrinogen; (U), uroporphyrinogen III decarboxylase; (C), coproporphyrinogen III oxidase.

Fig. 2. Patterns of porphyrin excretion in strains bearing single *hem12* and double *hem12 hem13-1* mutations

The bars represent the percentage of each porphyrin; the values in parentheses represent the total concentrations of porphyrins in nmol/g dry wt. Key to numbers: 8, 7, 6, 5, uro-, hepta-, hexa- and pentacarboxyporphyrin; 4, 3, 2, copro-, harderoporphyrin and protoporphyrin; i, dehydroisocoproporphyrin.

porphyrinogen oxidase activity) with the porphyrin patterns of strains bearing two mutations: in genes HEM12 and HEM13 (decreased decarboxylase activity and lack of oxidase activity).

We analysed both intracellular porphyrins and porphyrins excreted to the medium and we found that intracellular porphyrins from single (*hem12*) and double (*hem12 hem13-1*) mutant cells shared similar patterns: both single and the corresponding double mutants accumulated uro-, hepta-, hexa-, penta-, and coproporphyrin in similar proportions (results not shown). However, we did not find any traces of dehydroisocoproporphyrin, present in single mutants, in double mutants.

Surprisingly, patterns of porphyrins excreted to growth media were quite different between single and double mutants (Fig. 2). Double mutants excreted almost exclusively coproporphyrin and pentacarboxyporphyrin, but not dehydroisocoproporphyrin, whereas single mutants excreted uro-, hepta-, hexa-, penta-, copro-, dehydroisocopro- and harderoporphyrins, and protoporphyrin.

These results clearly indicate that the synthesis of dehydroisocoproporphyrin depends on the presence of an active coproporphyrinogen oxidase accompanied by an increased (in comparison to the wild-type) amount of pentacarboxyporphyrinogen. This confirms that the alternative route of metabolism of pentacarboxyporphyrinogen may function in mutants of *Saccharomyces cerevisiae* when impaired in uroporphyrinogen decarboxylase, and is one more proof of a close similarity of haem synthesis in yeast and humans.

Anna KURLANDZKA,* Teresa ZOLADEK,*
Joanna RYTKA* and Rosine LABBE-BOIS†

*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Rakowiecka 36, 02-532 Warszawa, Poland, and †Laboratoire de Biochimie des Porphyrines, Institut Jacques Monod, Université Paris VII, 2 Place Jussieu, F-75215 Paris, France

1. Rimington, C. (1989) *J. Clin. Chem. Clin. Biochem.* **27**, 473–486
2. Kappas, A., Sassa, A. & Anderson, K. E. (1984) in *The Metabolic Basis of Inherited Diseases* (Stanbury, J. B., Wyngarden, J. B., Frederickson, D. S., Goldstein, J. L. & Brown, M. S., eds.), pp. 1301–1384, McGraw-Hill Inc., New York
3. Nordman, Y. & Deybach, J.-C. (1990) in *Biosynthesis of Heme and Chlorophylls* (Dailey, H. A., ed.), pp. 491–541, McGraw-Hill Inc., New York
4. Elder, G. H. (1975) *J. Clin. Pathol.* **28**, 601–607
5. Elder, G. H. (1977) *Semin. Hematol.* **14**, 227–242
6. Elder, G. H. & Evans, J. O. (1978) *Biochem. J.* **169**, 205–214
7. Jackson, A. H., Lash, T. D. & Ryder, D. J. (1980) *Int. J. Biochem.* **12**, 775–780
8. Rytka, J., Bilinski, T. & Labbe-Bois, R. (1984) *Biochem. J.* **218**, 405–413
9. Kurlandzka, A., Zoladek, T., Rytka, J., Labbe-Bois, R. & Urban-Grimal, D. (1988) *Biochem. J.* **253**, 109–116
10. Kurlandzka, A. & Rytka, J. (1985) *J. Gen. Microbiol.* **131**, 2909–2918
11. Zagorec, M. & Labbe-Bois, R. (1986) *J. Biol. Chem.* **261**, 2506–2509
12. Zagorec, M., Buchler, J.-M., Treich, I., Keng, T., Guarente, L. & Labbe-Bois, R. (1988) *J. Biol. Chem.* **263**, 9718–9724
13. Mahler, H. R. & Lin, C. C. (1974) *Biochem. Biophys. Res. Commun.* **61**, 863–868
14. Labbe-Bois, R. & Volland, C. (1977) *Arch. Biochem. Biophys.* **179**, 565–577

Received 9 July 1990