Over-Production of Porphyrins and Heme in Heterotrophic Bacteria

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In *Escherichia coli*, *Pseudomonas aeruginosa*, and *Achromobacter metalcaligenes* δ-aminolevulinic acid synthase and δ-aminolevulinic acid dehydratase appear to be the rate limiting enzymes of porphyrin and heme biosynthesis. Bypassing of these enzymes by addition of δ-aminolevulinic acid or porphobilinogen results in overproduction of tetrapyrroles.

Comparative studies of porphyrin and heme biosynthesis indicate that δ-aminolevulinic acid synthase (EC 2.3.1.37) is the rate limiting enzyme in this biosynthetic pathway in all microorganisms tested so far [1–3]. There is little information about the regulatory function of δ-aminolevulinic acid dehydratase (EC 4.2.1.24), which has been isolated from *Saccharomyces cerevisiae* [4], *Propionibacterium shermanii* [5], and *Rhodopseudomonas spheroides* [6]. In the present study concentrations of porphyrins and heme were determined in *Escherichia coli*, *Pseudomonas aeruginosa*, and *Achromobacter metalcaligenes* following biosynthesis from the direct precursors of uroporphyrinogen, either δ-aminolevulinic acid (ALA) or porphobilinogen (PBG). Thus the corresponding enzymes, which might have a regulatory function in the tetrapyrrole synthesis of heterotrophic bacteria, were bypassed.

Sources of the organisms *E. coli*, *Ps. aeruginosa*, and *A. metalcaligenes* as well as the composition of a defined medium have been reported previously [7]. Supplements added to the medium in various experiments are given in the legends to the tables. ALA was obtained from Serva, Heidelberg, and PBG produced by *Propionibacterium shermanii* [9] was the kind gift of Dr. G. Müller, University of Stuttgart. All cultures were grown in 500 ml Erlenmeyer flasks at 37 °C with moderate aeration for up to 42 hours [1]. Cell density (dry weight/ml) was determined by turbidity measurement [9].

Porphyrins and heme were measured spectrophotometrically as methyl esters after separation by silica gel thin-layer chromatography [10]. Protoheme was converted into its pyridine hemochrome and assayed by difference spectrophotometry [11]. Small amounts of porphyrin esters were analysed as copper chelates [12] or by fluorescence measurement [13].

The three species synthesised heme and all porphyrins in the biosynthetic chain in varying amounts, as summarized in the Tables. The supplements had no significant influence upon growth of the cells.

The results reported in Table I indicated, that during biosynthesis from DL-lactate the porphyrin patterns of *E. coli* and *Ps. aeruginosa* are similar in that coproporphyrin is the main component formed in each, whereas *A. metalcaligenes* synthesised predominantly protoporphyrin. Addition of ALA or PBG elevated porphyrin and heme biosynthesis considerably, but in all cultures overproduction from ALA appeared to be greater than from PBG (Table II, III).

Iron(III) citrate (10 μM) enhanced heme formation in all culture systems about 2-fold, whereas total porphyrin synthesis both from DL-lactate and ALA was reduced by exogenous iron by about 40% (Table I, II). Porphyrin synthesis from PBG was not reduced by iron (Table III). In addition iron also altered the ratio of copro-/protoporphyrin as described previously [7].

Table I. Porphyrin and heme biosynthesis from DL-lactate with and without addition of iron(III) citrate (10 μM) in *Escherichia coli* (1), *Pseudomonas aeruginosa* (2), and *Achromobacter metalcaligenes* (3). T = traces.

Organism	Addition of iron	Heme [pmol/mg]	Total porphyrins [pmol/mg]	Distribution of porphyrins [%] Uro-	7 CO_2H-	6 CO_2H-	5 CO_2H-	Copro-	3 CO_2H-	Proto-
1	–	21	60	15	11	6	9	41	8	10
	+	38	28	12	4	T	2	33	10	39
2	–	14	157	17	10	6	8	49	5	5
	+	32	63	14	4	2	6	47	8	19
3	–	28	38	10	7	2	8	19	16	38
	+	66	18	7	1	T	T	49	17	26

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Table II. Porphyrin and heme biosynthesis from δ-aminolevulinic acid (0.2 mM) with and without addition of iron(III)-citrate (10 μM) in *Escherichia coli* (1), *Pseudomonas aeruginosa* (2), and *Achromobacter metalcaligenes (3)*.

Organism	Addition of iron	Heme [pmol/mg]	Total porphyrins [pmol/mg]	Distribution of porphyrins [%] Uro-	7 CO_2H-	6 CO_2H-	5 CO_2H-	Copro-	3 CO_2H-	Proto-
1	−	69	607	36	13	3	7	33	3	5
	+	116	446	41	10	5	8	27	3	6
2	−	61	937	42	12	3	5	35	1	2
	+	109	686	49	9	2	3	31	2	4
3	−	82	450	38	7	6	8	17	7	17
	+	149	283	31	5	2	4	41	8	9

Table III. Porphyrin and heme biosynthetsis from porphobilinogen (0.1 mM) with and without addition of iron(III) citrate (10 μM) in *Escherichia coli* (1), *Pseudomonas aeruginosa* (2), and *Achromobacter metalcaligenes* (3).

Organism	Addition of iron	Heme [pmol/mg]	Total porphyrins [pmol/mg]	Distribution of porphyrins [%] Uro-	7 CO_2H-	6 CO_2H-	5 CO_2H-	Copro-	3 CO_2H-	Proto-
1	−	43	370	33	7	3	8	42	3	4
	+	80	356	36	3	2	6	36	4	13
2	−	39	419	34	8	4	7	38	4	5
	+	68	397	40	7	2	5	29	4	13
3	−	55	287	36	9	3	5	14	10	23
	+	99	297	31	9	2	8	24	10	16

The formation of smaller amounts of porphyrin and heme from PBG than from ALA might be due to the rate of passage of the different molecules through the membranes. The amount of endogenous heme appears to be closely related to the iron concentration in the culture fluid [7]. The observation that porphyrin formation from DL-lactate drops when endogenous heme concentrations is increased and that tetrapyrrole synthesis is enhanced by addition of ALA favours ALA-synthase as the rate limiting enzyme in this biosynthetic chain [3, 14]. During incubation with exogenous ALA porphyrin formation is also depressed by higher heme levels, whereas synthesis from PBG appears to be unaffected by heme. This suggests repression of ALA-dehydratase by heme, which has also been observed in photosynthetic bacteria [6]. It is conceivable too that ALA-dehydratase is repressed by high concentrations of ALA, as has been shown in enzyme preparation from *Rhodopseudomonas spheroides* [15], although the physiological significance of this observation is difficult to assess.

The present results suggest that, in analogy to tetrapyrrole biosynthesis in phototrophic bacteria [3, 6], regulation in the heterotrophic bacteria of the overall synthesis seems to be associated with ALA-synthase and ALA-dehydratase. The effect of iron, which alters the copro-/protoporphyrin ratio, might be due to species-specific differences and appears to be of secondary nature.

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