Excretion of Porphyrins by Bacteria

Most microorganisms and animal cells excrete very small, biologically insignificant amounts of porphyrinogens (plus porphyrins), together with their precursors porphobilinogen and δ-aminolevulinic acid (ALA), which for reasons of economy are not utilized further. This fact by itself suggests that the synthetic pathway which culminates in heme is subject to strict molecular control. The key enzyme is ALA synthetase. ALA is the rate-limiting metabolite in the biosynthesis of porphyrins, and the prosthetic group of the cytochromes. In order to overcome the limiting factors in porphyrin synthesis, a high concentration of ALA is required; it can be supplied endogenously by induction of the synthetase, or supplemented exogenously. In the second case, i.e., when ALA is added, the regulative function of the synthetase is bypassed, and the total porphyrin synthesis is largely dependent on the available substrate. However, the cells are not capable of utilizing the greater portion of the excess porphyrinogens formed; this is largely independent of the availability of sufficient iron for synthesis of heme proteins. The cells avoid unlimited accumulation of porphyrinogens and porphyrins by excreting these substances. This is equally true of microorganisms[1,2] and of animal cells, e.g., liver cells in porphyria cutanea tarda in man[3], or chick embryo liver cells growing in culture after induction[4].

For the following study on the excretion of porphyrins in limited and unlimited porphyrin synthesis, 3 species of bacteria were chosen: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Achromobacter metalcaligenes*.

Experimental. The sources of these organisms have been listed in a previous paper[5]. They were grown on an ammonium-lactate-mineral medium[4] without supplemental iron. In order to ensure unlimited porphyrin synthesis, a concentration of 0.3 m*M* of ALA was chosen. Cultures of 150 ml each were grown in 500-ml Erlenmeyer flasks under moderate aeration[2] for up to 40 h. Cells and culture fluid were analyzed separately[2], whereby the porphyrins and heme were determined as their methyl esters[4].

Results. Table I shows the different patterns of porphyrins produced by *E. coli*, *Ps. aeruginosa*, and *A. metalcaligenes* grown with endogenous or exogenous ALA; lactate was the sole source of energy. Supplemental ALA stimulated porphyrin and heme synthesis in all 3 organisms, whereby the mode of utilization of available ALA varies according to the primary enzymatic equipment of each organism. *Ps. aeruginosa*, whose capacity for porphyrin production from endogenous ALA is greater than that of *E. coli* or *A. metalcaligenes*, also shows the highest rate of porphyrin synthesis from exogenous ALA. Uroporphyrin is the main product formed in the presence of added ALA. Of interest is the appearance of considerable quantities of the porphyrins with 7, 6, and 5 carboxyl groups. More than half of the porphyrins found in the fluid are excreted in their reduced form. This can be inferred from their subsequent oxidation during intensive aeration of the cells in the course of harvesting, as well as from the effect of acid, and is responsible for the intensified fluorescence following such treatment. Proceeding from the data in Table I, the intra- and extracellular percent distributions of the porphyrins are presented in Table II. Normally the organisms excrete practically no protoporphyrin into the medium. In synthesis from endogenous ALA they release only coproporphyrin (*E. coli* and *A. metalcaligenes*) and higher carboxylated porphyrins (*Ps. aeruginosa*). When ALA is available in excess for porphyrin synthesis (Table I), all porphyrin species, including porphyrinogens, appear in the medium. Whereas over 97% of the uroporphyrin is excreted, 78–93% of the porphyrins with 7 to 4 carboxyl groups, and as much as 50% of the tricarboxylic porphyrins, are excreted; the only porphyrin which can be utilized in other biosyntheses is protoporphyrin, which is lost to the extent of only 15–27%. Similarly, only up to 30% of the heme is passed into the medium, although its rate of synthesis increases 3- to 5-fold in the presence of exogenous ALA; if only endogenous ALA is available, about 17% is excreted.

The transport of porphyrins and heme involves macromolecules, onto which the porphyrins are bound by means of their carboxylic acid side chains. The culture fluids in porphyrin accumulation are rich in lipoprotein. In culture fluid from *A. metalcaligenes*, protoporphyrin was found to be bound to a macromolecular fraction[6]. Thin-layer chromatography and gas chromatographic analysis[7] of the lipid component of the macromolecules present in the supernatant of cultures of *A. metalcaligenes* has shown it to contain a mixture of waxes, consisting

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Table I. Biosynthesis of porphyrins and heme from endogenous and exogenous ALA in *E. coli*, *Ps. aeruginosa*, and *A. metalcaligenes*

Organism	Addition of ALA	Heme pMol/mg	Total porphyrins pMol/mg dry weight	Percent distribution of porphyrins						
				8 COOH	7 COOH	6 COOH	5 COOH	4 COOH	3 COOH	2 COOH
E. coli	—	18.7	38.6	8.7	3.6	1.3	2.3	52.0	8.1	24.0
	+	61.9	724.5	34.2	12.2	4.3	7.7	21.5	8.5	11.6
Ps. aeruginosa	—	10.5	141.5	6.1	3.5	1.6	14.8	65.0	4.3	4.7
	+	51.0	1822.5	61.0	8.1	3.5	8.3	14.2	2.5	2.4
A. metalcaligenes	—	24.7	26.2	8.0	5.0	1.2	2.5	22.1	15.3	45.9
	+	79.2	707.8	35.8	12.8	4.6	9.7	14.2	8.3	14.6

Table II. Excretion of porphyrins and heme from the cells

Organism	Addition of ALA	Heme	Total porphyrins	Porphyrins according to the biosynthetic chain						
				8 COOH	7 COOH	6 COOH	5 COOH	4 COOH	3 COOH	2 COOH
		Percentage found in the culture fluid.								
E. coli	—	15.4	17.8	–	–	–	–	73.2	–	–
	+	29.1	71.2	91.7	76.4	77.9	76.4	86.8	49.3	24.3
Ps. aeruginosa	—	16.4	58.4	66.4	38.5	–	75.4	88.2	43.2	–
	+	27.5	89.3	96.4	86.4	87.8	85.3	89.7	53.5	16.7
A. metalcaligenes	—	18.5	16.3	–	–	–	–	70.7	–	–
	+	26.7	69.8	91.6	73.8	78.5	76.9	84.3	50.7	24.5

mainly of saturated alcohols and fatty acids with chain lengths of C_{14}, C_{16}, and C_{18}[8].

Discussion. The excretion of coproporphyrin-III under conditions of iron deficiency was observed in various bacteria, especially in *Corynebacterium diphtheriae*[9]. The medium for *E. coli*, *Ps. aeruginosa*, and *A. metalcaligenes* in the experiments under discussion here likewise contained iron concentrations of less than 0.1 μM. Supplemental iron (10 μM) enhances the production of heme by 50–100%, and reduces porphyrin synthesis by about 40%[5]. However, it has no influence on the relative distributions of the individual porphyrins between the cells and the medium.

The elimination of coproporphyrin in controlled porphyrin synthesis is of general biological interest: Microorganisms, mammals, and man excrete predominantly coproporphyrin, along with smaller amounts of the porphyrins bearing 8 to 3 carboxyl groups, all of which remain in their reduced forms during heme biosynthesis. Microorganisms and animal cells show analogous behavior when porphyrin synthesis can proceed without limitation, i.e., in the presence of added ALA, or of increased activity of ALA synthetase. The greater portion of the porphyrins with 8 to 4 carboxyl groups, and over 90% of the uroporphyrin, are eliminated from the cells (congenital porphyria[10] and porphyria cutanea tarda[3]). Even protoporphyrin is excreted (erythropoietic protoporphyria and porphyria variegata[10]), if, as is the case with microorganisms to which ALA is added, more protoporphyrin is produced than can be utilized in heme synthesis. The pattern of distribution of porphyrins with 8 to 5 carboxyl groups following synthesis from added ALA is strikingly similar to that found in chronic hepatic porphyria[3], which suggests the existence of a similar enzymatic mechanism of stepwise decarboxylation of uroporphyrinogen in both types of cells.

The elimination of 70–90% of the total porphyrins formed from exogenous ALA in *E. coli*, *Ps. aeruginosa*, and *A. metalcaligenes* must probably be regarded as a mechanism for the protection of the cell, whose normal processes would most likely be disrupted if extremely large amounts of photoactive porphyrin carboxylic acids were to accumulate. These experiments illustrate once again the suitability of microorganisms as model systems, especially for the study of the principles underlying the excretion of porphyrins, which apparently are of general validity.

Zusammenfassung. Escherichia coli, *Pseudomonas aeruginosa* und *Achromobacter metalcaligenes* bilden sämtliche, der Biosynthesekette zum Häm entsprechenden Porphyrine aus endogener δ-Aminolävulinsäure (ALS) und scheiden Koproporphyrin und Häm aus. Porphyrinsynthesestimulation mit exogener ALS führt hingegen zur Elimination auch der übrigen Porphyrine aus den Organismen, wobei die höher carboxylierten zu 78–99% in die Kulturflüssigkeit übergehen und Protoporphyrin noch zu über 75% in den Zellen verbleibt.

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[11] This work was supported by grants from the Deutsche Forschungsgemeinschaft.

Regionale Inkorporation der optischen Isomeren der Aminosäure Prolin-H³ im Autoradiogramm des Mäusegehirns[1]

D,L-Prolin wird im Gegensatz zu anderen Aminosäuren in verschiedenen Hirnregionen der Maus sehr unterschiedlich eingebaut[2–4]. Das Enzym D-Aminosäure-Oxydase weist im Gehirn der Maus[5] und anderer Säugerspecies[6] ebenfalls beträchtliche regionale Aktivitätsunterschiede auf. Daher erscheint ein Vergleich der topographischen Verteilung dieses Enzyms mit dem Einbauverhalten der optischen Isomeren des Prolins im Gehirn von Interesse.

Männliche Mäuse des Stammes NMRI/Han. erhielten im Alter von 12 Wochen je 20 μCi/g Körpergewicht D,L-