THE EFFECT OF DL-LACTATE ON REGULATION OF PORPHYRIN AND HEME BIOSYNTHESIS IN *ESCHERICHIA COLI* AND *ACHROMOBACTER*

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1. Introduction

Porphyrin and heme biosynthesis in microorganisms and liver cells is affected by physiological factors such as oxygen tension [1, 2], nutrition [1], iron concentration [3], and presence of direct precursors of δ-aminolevulinic acid (ALA) such as glycine [4]. Alteration of environmental conditions forces considerable adaptive changes in regulation of biosynthesis in the cell. The main control mechanism is thought to be δ-aminolevulinic acid synthase, the formation and activity of which is dictated by the end product, heme [5]. Bypassing the function of ALA synthase by addition of exogenous ALA provokes a 10- to 20-fold increase of total porphyrin concentration in bacteria [6]. This communication deals with the changes in biosynthesis from a single energetic substrate, DL-lactate.

2. Experimental procedure

Sources of *Escherichia coli* and *Achromobacter metalcaligenes* were reported in a previous paper [3].

Cells for resuspension were cultivated in Trypticase Soy Broth (Baltimore Biological Laboratory, Baltimore, Maryland, USA), and incubated in cultures of 450 ml in 2 litre Fernbach flasks at 37°C for 18 hr in a horizontally shaking incubator (amplitude 50 mm, frequency 60/min). After the cells had been sedimented (6000 *g*, 5°C, 30 min) and washed twice in 0.85% NaCl solution they were resuspended at a density of 1.2 mg/ml in a defined medium containing DL-lactate 40 mmoles/l, NaCl 86 mmoles/l, $MgSO_4 \cdot 7\,H_2O$ 0.81 mmoles/l, $(NH_4)H_2PO_4$ 8.7 mmoles/l, K_2HPO_4 5.7 mmoles/l, $MnSO_4 \times 4\,H_2O$ 0.5 μmole/l; pH 6.9. The suspensions were divided into 30 ml portions, which were transferred to 100 ml Erlenmeyer flasks and incubated for 20 hr at 37°C under constant agitation (see above). Porphyrins and heme were measured spectrophotometrically as methyl esters after separation by silica gel thin-layer chromatography [7]. Protoheme was converted into its pyridine hemochrome and assayed by difference spectrophotometry [8]. Small amounts of porphyrins were measured as copper chelates [9].

For preparation of a cell-free extract for enzyme analysis the sedimented cells (20 000 *g*, 5°C, 15 min) were washed in Na–K–phosphate buffer (Sorensen) 67 mmoles/l (pH 7.0) and after repeated centrifugation suspended in Tris buffer (40 mmoles/l, pH 7.4) at a density of 20 mg/ml. The cells were disintegrated in an MSK cell homogenizer (B. Braun-Apparatebau, BRD-3508 Melsungen) with intermittent cooling by a stream of CO_2. After centrifugation (20 000 *g*, 0°C, 10 min) the protein concentration of the supernatant was determined by the biuret reaction, using Labtrol as a standard [10], and adjusted to 15 mg/ml. Lactate dehydrogenase activity (EC 1.1.1.27) was determined according to Bergmeyer and Bernt [11].

3. Results

The results of experiments with cells of *Achromobacter metalcaligenes* grown in Trypticase Soy Broth

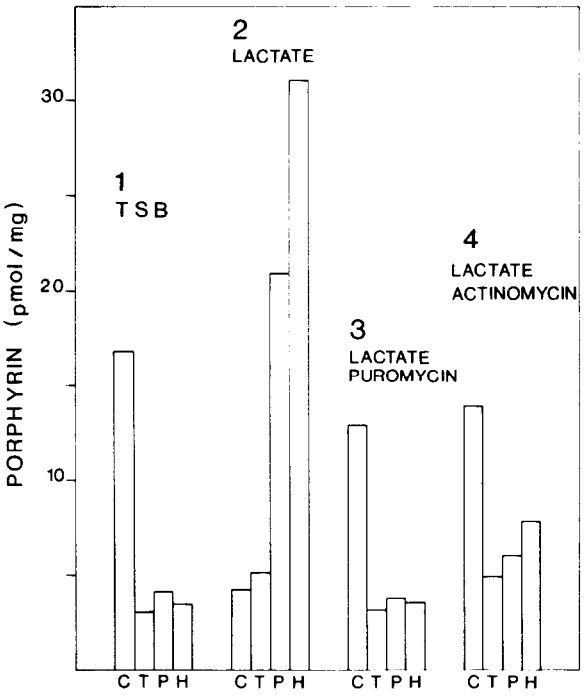

Fig. 1. Effect of lactate on porphyrin and heme synthesis in resuspended cells of *Achromobacter metalcaligenes*. Cells were cultivated first in Trypticase Soy Broth (TSB) and after sedimentation resuspended in TSB (1), lactate (2), lactate with addition of puromycin (30 μg/ml) (3), lactate with addition of actinomycin D (15 μg/ml) (4). Tetrapyrrole concentrations in the cells before resuspension were 7 pmoles/mg cells for coproporphyrin (C). 3 pmoles/mg cells for tricarboxyporphyrin (T), 3 pmoles/mg cells for protoporphyrin (P), and 3 pmoles/mg cells for heme (H).

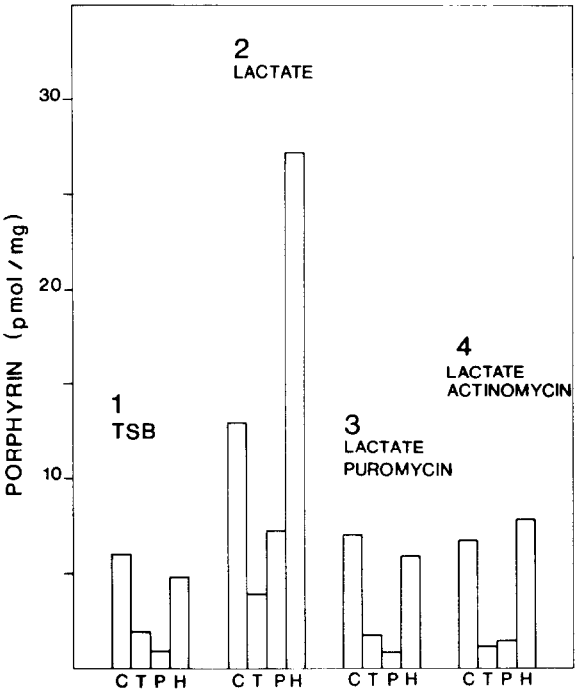

Fig. 2. Effect of lactate on porphyrin and heme synthesis in resuspended cells of *Escherichia coli*. Cells were cultivated first in Trypticase Soy Broth (TSB) and after sedimentation resuspended in TSB (1), lactate (2), lactate with addition of puromycin (30 μg/ml) (3), lactate with addition of actinomycin D (20 μg/ml) (4). Tetrapyrrole concentrations in the cells before resuspension were 4 pmoles/mg cells for coproporphyrin (C), 1.5 pmoles/mg cells for tricarboxyporphyrin (T), 1 pmole/mg cells for protoporhyrin (P), and 4 pmoles/mg cells for heme (H).

(TSB) and resuspended in the defined lactate-mineral medium are given in fig. 1, together with data from those grown in TSB for comparison. In the cells resuspended in lactate-medium a considerable increase in heme and porphyrin synthesis was found; heme levels increased to 10-fold, and protoporphyrin increased 5-fold. The cells resuspended in TSB as well as the cells without resuspension exhibited a higher content of coproporphyrin. The stimulating effect of lactate on porphyrin and heme biosynthesis was inhibited with puromycin and actinomycin D. After addition of these inhibitors to the lactate-mineral medium, total porphyrin and heme concentrations as well as the porphyrin patterns in the cultures were found to be similar to those of the cultures grown in TSB.

In the same manner, porphyrin and heme synthesis was also stimulated by lactate in *Escherichia coli*, and was inhibited by addition of puromycin or actinomycin D (fig. 2). In both organisms increasing concentrations of tricarboxylic porphyrin and protoporphyrin were observed, but in *E. coli* coproporphyrin remained the dominant component.

Parallel experimental were set up to test the activity of lactate dehydrogenase (LDH). After resuspension in lactate-mineral medium and incubation for 20 hr the enzyme activity was about six times higher than in the cells resuspended in TSB (table 1). This increase of activity was suppressed by puromycin.

Table 1
Lactate dehydrogenase activity in resuspended cells of *Achromobacter metalcaligenes*.

Medium for resuspension	Additions	LDH activity (m units/mg protein) Experiment I	Experiment II
TSB	–	178	134
Lactate	–	1320	915
Lactate	Puromycin (30 μg/ml)	374	335

Cells were grown first in Trypticase Soy Broth and resuspended after sedimentation (see Experimental procedure).

4. Discussion

The biosynthetic pathway of porphyrins is closely related to both the tricarboxylic acid cycle and to synthesis and function of the cytochromes. The bacterial cell cultivated in TSB has at its disposal a variety of compounds, which can be used both in synthetic processes and as sources of energy. Growing in a defined mineral salt medium containing a single energetic substrate, the cell derives the energy it needs for anabolic and for catabolic metalolism from respiratory chain-linked phorphorylation. With lactate as the the sole substrate the cell is forced to resort to lactate respiration, which is linked to the cytochrome system [12]. Under these conditions the synthetic role of the tricarboxylic acid cycle enzymes involved in both anabolic and catabolic processes would predominate, as has been shown for liver cells [13, 14].

The higher levels of such physiologically useful metabolites as heme and protoporphyrin, which serve as direct precursors of the cytochromes, indicate the strict regulation occurring in cells grown in lactate. This is in contrast to the higher levels of side products such as coproporphyrin found in cells grown in TSB, where they are mainly excreted into the culture medium [15].

As the effect of lactate could be suppressed by inhibitors of nucleic acid and protein synthesis, the enhanced production of porphyrin and heme can be interpreted as neosynthesis of specific enzymes, i.e., induction. Though this is demonstrated only for LDH, it seems justifiable to conclude that additional induction of ALA synthase occurs, as the porphyrin levels can be regarded as an indirect measure of ALA synthase [4, 8, 16]. The question remains open as to whether the increase of the LDH activity might indicate the presence of a separate, inducible fraction of the enzyme.

Measurement of ALA synthase activity and determination of percentage distribution of the porphyrin isomers also await further investigation.

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