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Aeration-dependent changes in composition of the quinone pool in *Escherichia coli*

Evidence of post-transcriptional regulation of the quinone biosynthesis

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Abstract The aeration-dependent changes in content of various quinones in *Escherichia coli* were found to be unaffected by a prokaryotic translation inhibitor chloramphenicol. In addition, this process was shown to be completely intact in cells with mutated *fnr*, *arc* and *appY* loci. It is assumed that *E. coli* possesses a special system of oxygen-dependent post-transcriptional regulation of the quinone biosynthetic pathways.

Key words: Arc; Fnr; AppY; Quinone biosynthesis; Ubiquinone; Menaquinone; Aerobiosis; Anaerobiosis; *Escherichia coli*

1. Introduction

It is generally assumed that the O_2-dependent control of levels of various respiratory chain components mainly occurs via two pleotropic transcription regulation systems, i.e. Arc and Fnr [1]. This regulatory duet has been recently replenished with one more regulatory system, AppY, which takes part in aeration-dependent expression of *hya* operon (encoding for hydrogenase I), and *appA* operon (encoding for an acid phosphatase and the third putative terminal oxidase of *Escherichia coli* with large sequence similarities to the *bd* type oxidase) [2–5].

Composition of the *E. coli* quinone pool is also known to dramatically change during aerobiosis-to-anaerobiosis transition in correlation with coherent changes in content of respiratory chain enzymes [6]. With three major quinones synthesised in *E. coli* (two naphthoquinones: MQ_8, DMQ_8 and a benzaquinone Q_8) [7], anaerobic electron transport utilising TMAO, DMSO and fumarate as acceptors of reducing equivalents is accompanied by a consistently high content of MQ_8 and DMQ_8, while Q_8 is predominant during aerobic growth where MQ_8 content is essentially low [8–10]. The pattern of this aeration-dependent switching in quinone content resembles that of the Fnr, Arc and AppY-regulated respiratory enzymes. Unden [11] earlier has ruled out the possibility of Fnr regulation of biosynthesis of naphthoquinones, but the involvement of Arc and AppY in the process of quinone switching remained unknown. In this paper we investigated the aeration-dependent quinone biosynthesis regulation and the role of Arc, Fnr and AppY in this process.

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Abbreviations: DMQ_8, demethylmenaquinone-8; DMSO, dimethylsulfoxide; MQ_8, menaquinone-8; TMAO, trimethylamine *N*-oxide; Q_8, ubiquinone-8

2. Methods

2.1. Bacterial strains

The *E. coli* strains used are listed in Table 1: ECL, AN and TC series strains were kindly provided by Professor E.C.C. Lin, Professor I.G. Young and Professor T. Atlung, respectively.

2.2. Growth media and conditions

E. coli cells were grown in M9 medium supplemented with 0.05% yeast extract, 0.1% tryptone, 0.4% maltose at pH 7.4 and 37°C. All strains were inoculated to 3×10^7 cells/ml. Anaerobic batch cultures were grown for 14–18 h up to a density of 1.2×10^9 cells/ml in 500 ml glass flasks completely filled up and sealed with tape. Aerobic batch cultures were incubated up to $4.8–6\times10^8$ cells/ml in 1 l glass flasks with 50 ml of growth media vigorously shaken at 200 r.p.m.

For chloramphenicol treatment aerobic and anaerobic cells were pre-grown in M9 medium to $4.8–6\times10^8$ cells/ml (the early middle exponential growth phase) and 1.2×10^9 cells/ml density, respectively. The culture was then kept for 30 min at 0°C and diluted to a 1.2×10^8 cells/ml with the same medium containing chloramphenicol (100 μg/ml). Further incubation proceeded under the above-described growth conditions.

2.3. Extraction of quinones

After growth, cells were collected by centrifugation, washed once with equal volume of M9 growth medium without substrates, tryptone and yeast extract and once with 5% sucrose. The pellet was stored at −20°C for up to 1 week.

Quinones were extracted from bacterial samples (70 mg of cell protein), using a modified Unden's procedure [11]. (a) Two milliliters of methanol and 2 ml of hexane were added to the sample. Pellet was thoroughly mixed and 2 ml of acetone was added with subsequent mixing of suspension. (b) After centrifugation of probe at $3000\times g$ for 10 min, the upper quinone-containing hexane fraction was collected. (c) Three milliliters of hexane was added to the residue, the suspension was mixed and step (b) was repeated. (d) Hexane fractions were combined, 3–4 crystals of ferricyanide were added to fully oxidise quinones and probe was washed once with equal volume of methanol/water (95:5). (e) Hexane fractions were removed, rapidly evaporated under a stream of air at 40°C and dissolved in eluent (acetonitryl/isopropanol, 60:40).

2.4. Chromatography of quinones

Bacterial quinones were separated by a reverse-phase HPLC technique using an Ultrasphere-ODS 250×4.6 mm, 5 mm column (Altex, USA) on Beckman 344 (USA) chromatograph with Pye Unicam 4021 dual channel detector (UK). A mixture of acetonitryl/isopropanol (60:40) was used as eluent (the flow, 1 ml/min). The naphthoquinone and benzoquinone detection was simultaneously carried out at 270 and 328 or 260 and 328 nm. Compounds were collected and characterised using a Hitachi 557 spectrophotometer (Japan) at 240–360 nm by comparing spectra of the studied samples with those derived from *E. coli* strains lacking either naphthoquinones (AN386) or benzaquinone (AN385). Quinone fractions from the same strains were used to

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estimate the retention time (t_R). The following t_R values were achieved: Q_8, 7.7 min; DMQ_8, 10.3 min; MQ_8, 11.5 min. Standards of Q_{10} and vitamin K_1 were used for quantification. Quinone content was determined by calculating from elution band area of standards according to calibration curves plotted with respect to the molar extinction coefficients of substances in petrol ether at 269–270 nm [7].

β-Galactosidase activity was measured as described previously [15]. Protein content was determined by a modified biuret micro-method.

3. Results and discussion

In line with observations of Collins and Jones [7], we found that the quinol pool in aerobically grown *E. coli* comprises to approximately 65% Q_8 and 32% DMQ_8 with 2–4% left to MQ_8. A switch to anaerobiosis was followed by an 8-fold decline in Q_8 and up to a 20-fold increase in MQ_8 content, whereas the level of DMQ_8 was elevated 1.5–2-fold, the overall content being 7%, 42% and 51% for Q_8, DMQ_8 and MQ_8, respectively (Fig. 1). The rates seemed to be similar among different strains, although the total quinol content varied up to 1.6-fold for Q_8 and 2-fold for DMQ_8 with remarkable stability for MQ_8 (Table 2).

Deletions in *arcA*, *arcB*, *fnr* and *appY* genes did not result in any significant changes (Table 2). From these data one could either assume that (i) an unknown fourth transcription-regulating system is involved in the observed switching in quinone content, or (ii) the observed quinone switching occurs at the post-transcriptional level as a result of the enzymatic activity modulation in the menaquinone and ubiquinone biosynthetic pathways. To put these assumptions to the test, in the next series of experiments we examined the effect of a transcription inhibitor chloramphenicol. To our surprise the inhibitor totally failed to repress the aerobiosis-induced increase in [Q_8] (Fig. 2A) as well as of anaerobiosis-induced increase in [MQ_8] (Fig. 2B). On the other hand, under the same conditions chloramphenicol completely arrested growth and the anaerobic induction of cytochrome *bd* which is known to be regulated at the level of transcription by Arc and Fnr systems [16]. The level of cytochrome *bd* was monitored by measuring β-galactosidase activity in the *E. coli* ECL942 strain with a *lacZ–cyd* fusion (Fig. 2C).

The above results suggest that, irrespective of aeration conditions, the *E. coli* cells (1) house a full set of enzymes required for the biosynthesis of either MQ_8 or Q_8 and that (2) the O_2-dependent quinone switching occurs at the post-tran-

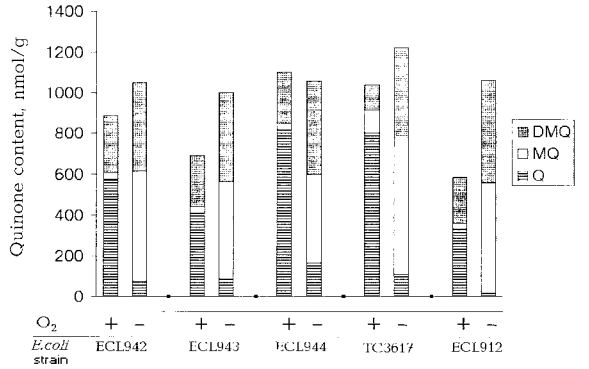

Fig. 1. Ubiquinone (Q_8), menaquinone (MQ_8) and demethylmenaquinone (DMQ_8) content in aerobically and anaerobically grown *E. coli*: ECL942, w.t.; ECL943, Δ*arcA*; ECL944, Δ*arcB*; TC3617, Δ*appY*; ECL912 Δ*arcB*, Δ*fnr*.

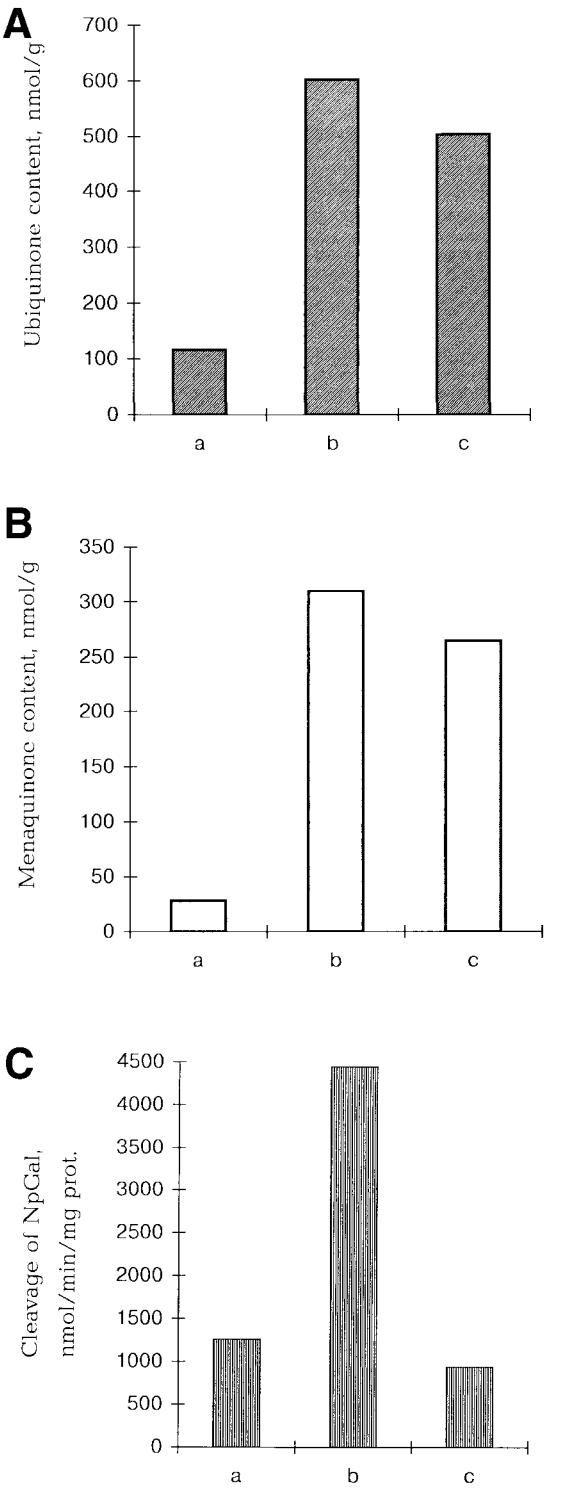

Fig. 2. The effect of chloramphenicol on ubiquinone (A), menaquinone (B) and cytochrome *bd* levels (C) in *E. coli*. A: Cells were pre-grown anaerobically for 18 h (a) with subsequent shift to aerobic growth conditions (b), or aerobiosis plus 100 μg/ml chloramphenicol (c) for 4 h. B: Cells were pre-grown aerobically for 4 h (a) with subsequent aerobiosis-to-anaerobiosis shift (b), or anaerobiosis plus 100 μg/ml chloramphenicol (c) for 4 h. C: Cells were pre-grown aerobically for 4 h (a) with subsequent shift to anaerobic growth conditions (b), or anaerobiosis plus 100 μg/ml chloramphenicol (c) for 4 h.

Table 1
Bacterial strains

E. coli strains	Genotype	Reference
ECL942	*cyd*$^+$ϕ*(cyd-lac)bla*$^+$	
ECL943	ECL942; *arcA*1 *zjj*::Tn10	[12]
ECL944	ELC942; *arcB*1 *zjj*::Tn10	
ECL901	ϕ*(IctD-lac)* Str^r	
ECL912	ϕ*(IctD-lac) arc*B1 *fnr-1 zci*::Tn10	[13]
ECL915	ϕ*(sdh-lac) fnr-1 zci*::Tn10 Str^r	
AN385	*F*$^-$ *thi ubi420*$^-$ *men*$^+$ Str^r	
AN386	*F*$^-$ *thi ubi*$^+$ *menA401*$^-$ Str^r	[14]
TC3617	*appY*::*aphA-3561*	
TC3594	Δ*(appY-ent)*	[5]

Table 2
The aerobic-to-anaerobic ratios for three *E. coli* quinones

E. coli strains	Aerobic-to-anaerobic quinone ratios		
	Q_8	DMQ_8	MQ_8
ECL942 (wild type)	8	0.6	0.06
ECL943 (Δ*arcA*)	5	0.7	0.05
ECL944 (Δ*arcB*)	5	0.5	0.07
TC3617 (Δ*appY*)	7	0.7	0.17
ECL912 (Δ*nfr*, Δ*arcB*)	10	0.6	0.06

scriptional level via enzymatic activity modulation. Regulation at this level bypasses the Arc, Fnr and AppY influence. This possibility is emphasised by the fact that post-transcriptional regulation of the respiratory chain-linked components has not been previously reported in *E. coli*, and regulatory effects are believed to be engendered at the level of transcription through the RNA–polymerase activity modulation by DNA-binding modules of response regulating systems represented by Arc, Fnr and AppY [17].

In this context, observations by Gilbert et al. [18] should be mentioned. The authors have found that the expression of one of the ubiquinone biosynthetic genes (ubiG–lacZ) in cells grown anaerobically in the presence of nitrate was 4 times decreased comparing with aerobic glycerol-grown cells. It is not excluded that effects of this kind can alter kinetics of changes in the quinone pool rather than the final levels of the three studied quinones. These levels are, according to our data, controlled post-transcriptionally. Under the conditions used, the Arc, Fnr and AppY regulatory systems do not contribute to this process.

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