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Effect of histamine on the signal transduction of the AtoS–AtoC two component system and involvement in poly-(R)-3-hydroxybutyrate biosynthesis in *Escherichia coli*

D. A. Kyriakidis[1,2], **M. C. Theodorou**[1], **P. S. Filippou**[1], **K. D. Kyriakidis**[3], and **E. Tiligada**[3]

[1] Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece
[2] National Hellenic Research Foundation, Athens, Greece
[3] Department of Pharmacology, Medical School, University of Athens, Athens, Greece

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Summary. AtoS–AtoC two-component system acts directly on the *atoDAEB* operon transcription to regulate the biosynthesis of short-chain poly-(R)-3-hydroxybutyrate. This study sought to investigate the effect of histamine and compound 48/80 on the regulation of AtoS–AtoC two-component system in *Escherichia coli* K-12 MA255 (*speC*$^-$, *speB*$^-$) and the isogenic *E. coli* strains BW25113 (*atoSC*$^+$) and BW28878 (Δ*atoSC*) transformed with plasmids carrying related genes. Histamine or compound 48/80 induced or tended to reduce *atoC* transcription, respectively, while neither compound showed any effect on *atoDAEB* operon transcription. Moreover, histamine down-regulated poly-(R)-3-hydroxybutyrate biosynthesis, whereas compound 48/80 up-regulated its biosynthesis, maximal induction being obtained in the presence of multiple copies of AtoS–AtoC. Interestingly, co-administration of histamine counteracted this inductive effect of compound 48/80. The reported data provide the first evidence for a differential modulator role of histamine and compound 48/80 on the AtoS–AtoC two-component system signaling in potentially pathogenic bacteria, leading to a new perspective on their symbiotic behavior.

Keywords: AtoS–AtoC – Compound 48/80 – *Escherichia coli* – Histamine – Poly-(R)-3-hydroxybutyrate – Two-component system

Abbreviations: *atoSC*$^+$, genetic locus encoding the AtoS, AtoC proteins; Az, antizyme; C48/80, compound 48/80; cPHB, complexed poly-(R)-3-hydroxybutyrate; *E. coli*, *Escherichia coli*; GI, gastrointestinal; HI, histamine; HK, histidine kinase; ODC, ornithine decarboxylase; PHB, poly-(R)-3-hydroxybutyrate; RR, response regulator; SCFA, short-chain fatty acid; TCS, two-component system

Introduction

Histamine (HI) is a biogenic amine derived from histidine with multiple activities in inflammation, host defence against bacterial infections, neurotransmission, gastrointestinal and circulatory functions and cell proliferation (Novak and Falus, 1997; Hori et al., 2002; Akdis and Blaser, 2003; Kakavas et al., 2006). In addition to its well-established action though histaminergic receptors (Akdis and Blaser, 2003; Kakavas et al., 2006), an intracellular messenger role for HI with functional consequences in physiological and pathophysiological events has also been suggested (Novak and Falus, 1997). Amongst others, naturally occurring polyamines have been suggested to regulate mast cell secretion, while the interconnection between HI and the basic polyamine compound 48/80 (C48/80) in mast cell function has long been established (Ennis et al., 1980; Katsu et al, 1985).

In prokaryotic cells, HI and polyamines are reported as cellular components that enable pathogenic bacteria to survive and to overcome host defence mechanisms (Crosa and Walsh, 2002). In experimental peritonitis, HI modulated the defence reactions of HI-deficient mice against *Escherichia coli* infection (Hori et al., 2002). Moreover, bacteria, including *E. coli*, promote the development of the immune system in the gastrointestinal (GI) tract (Guarner and Malagelada, 2003), where HI is a key mediator and regulates gastric acid secretion and thus adaptation of pathogens, like *Helicobacter pylori*, to their microenvironment and to drug therapies often *via* two component systems (TCS) (Guarner and Malagelada, 2003; Kakavas et al., 2006).

Polyamines appear to modulate cellular functions in both prokaryotes and eukaryotes, at least though interaction with nucleic acids and protein synthesis (Igarashi and Kashiwagi, 2000), while their uptake and synthesis are

negatively regulated by antizyme (Az), a polyamine-inducible endogenous non-competitive protein inhibitor of the rate-limiting biosynthetic enzyme ornithine decarboxylase (ODC) (Kyriakidis et al., 1978; Lioliou and Kyriakidis, 2004). The cloning and sequencing of the *E. coli* Az gene (Canellakis et al., 1993) disclosed unexpectedly that Az has a second function as the response regulator (RR) of a TCS family (Hoch and Silhavy, 1993). In particular, it was shown that Az is identical to the gene product of *atoC* (Blattner et al., 1997) and therefore Az is now referred to as AtoC, with dual function as a transcriptional and post-translational regulator (Lioliou and Kyriakidis, 2004).

Upon induction, this TCS activates the expression of the *atoDAEB* operon (Lioliou and Kyriakidis, 2004; Lioliou et al., 2005), encoding for proteins involved in short-chain fatty acid (SCFA) catabolism (Jenkins and Nunn, 1987). This is an essential function of the symbiotic GI flora that digests unutilized energy substrates, like carbohydrates to SCFAs in favor of their host (Guarner and Malagelada, 2003). The transcriptional activation of the *atoDAEB* operon requires the phosphorylation of the AtoC RR by its autophosphorylated partner AtoS, the membrane-bound sensor histidine kinase (HK) of the TCS (Canellakis et al., 1993; Lioliou et al., 2005; Grigoroudis et al., 2007). In an ever changing microenvironment, cells maintain their homeostasis, survival and proliferation by the adaptive and protective cellular stress response (Tiligada et al., 2002). Among the innumerable adaptive responses elicited through TCSs in bacteria (Zhou et al., 2003), the regulation of the complexed poly-(R)-3-hydroxybutyrate (cPHB) biosynthesis by the direct action of the AtoS–AtoC TCS on the *atoDAEB* operon transcription has been demonstrated recently (Theodorou et al., 2006, 2007). The abundantly naturally occurring cPHB, is a ubiquitous constituent of prokaryotic and eukaryotic cells with valuable physiological functions, including amongst others its contribution in Ca^{2+} homeostasis (Reush et al., 1995).

The speculation that HI and polyamines may be involved in interplay regulated at the level of gene expression or metabolism remains largely elusive (Medina et al., 2003). In contrast to polyamines, a potential interaction between HI and nucleic acids has not received adequate attention. Interestingly, the histaminergic H_1 receptor antagonist chlorpheniramine, a 1,4 diamine, has been shown to bind to nucleic acids and to affect protein synthesis and ODC translation (Medina et al., 2003). Accordingly, the presence of the imidazole ring and the hydrophobic backbone with 1,4 positive charges could allow HI to interact with nucleic acids and proteins (Igarashi and Kashiwagi, 2000).

Consequently, this study aimed at investigating the effect of HI and the polyamine C48/80 on the signal transduction of the AtoS–AtoC TCS and their contribution to the regulation of cPHB biosynthesis by the AtoS–AtoC TCS in *E. coli*. The reported data provide the first evidence for a modulator role of HI and C48/80 on the AtoS–AtoC TCS signaling in this microorganism, leading to a new perspective on the symbiotic behavior of potentially pathogenic bacteria.

Materials and methods

Bacterial strains and plasmids

E. coli K-12 strain MA255 [F$^-$, *thr*$^-$, *leuB6*(Am), *fhuA2*?, *lacY1*, *glnV44*(AS)?, *gal-6*, λ$^-$, *relA1*?, *CanR*?-1, *speB*, *speC*, *rpsL133*(strR), χ*ylA7*, *mtlA2*, *thi*$^-$] was obtained from the *E. coli* genetic stock Center, Department of MCDB, Yale University, USA. *E. coli* K-12 BW25113 (*atoSC*$^+$) [*lac*I^q *rrn*B3 Δ*lac*Z4787 *hsd*R514 Δ(*ara*BAD)567 Δ(*rha*BAD)568 *rph*-1] (Haldimann and Wanner, 2001) and BW28878 (Δ*atoSC*) [*lac*I^q *rrn*B3 Δ*lac*Z4787 *hsd*R514 Δ(*ara*BAD)567 Δ (*rha*BAD)568 *rph*-1 Δ(*ato*SC)569] were a kind gift from Hirofumi Aiba (Laboratory of Molecular Microbiology, School of Agriculture, Nagoya University, Japan). All plasmids have been used as described previously (Canellakis et al., 1993; Theodorou et al., 2006, 2007; Filippou et al., 2007).

Plasmids pUC-Az (Fig. 1A), containing the *atoS*, *atoC* genes and a part of the *atoDAEB* operon (*atoD*, *atoA*, and two-thirds of *atoE*) and pCPC-Az (Fig. 1C), containing the *atoC* gene and the same part of the *atoDAEB* operon as pUC-Az, have been described previously (Canellakis et al., 1993). Plasmids pUC-Az(AtoC$^-$) (Fig. 1B) is a pUC-Az derivative that does not express the AtoC protein due to the frame shift mutation introduced at codon 28 (Theodorou et al., 2007).

Plasmid pCPG5 (*atoA-lacZ*) carries a 1.2 kb fragment [*Eco*RI (1761)-*Hin*cII (2943)] of plasmid pCPC-Az, containing the *atoDAEB* operon promoter region, the *atoD* gene and a part of *atoA* gene (−419 to +1186 bp region) fused to the promoterless *lacZ* gene on pMLB1034 vector (Fig. 1D). Plasmid pCPG6 (*atoC-lacZ*) carries a 1.337 kb fragment, containing a part of *atoS* gene, the *atoC* promoter region and 187 bp downstream of +1 of *atoC* gene (−1150 to +187 bp region), fused to the promoterless *lacZ* gene on pMLB1034 vector (Fig. 1E) (Filippou et al., 2007).

Growth conditions

E. coli were grown at 37 °C through to the post-logarithmic phase of growth, in M9 mineral medium, supplemented with 0.5% (w/v) glucose as the carbon source and 0.1 mM $CaCl_2$, 1 mM $MgSO_4$, 1.7×10^{-3} mM $FeSO_4$, 1 μg/ml thiamine and 80 μg/ml DL-proline (for BW25113 and BW28878 strains), or 50 μg/ml DL-threonine (for MA255 strains). Putrescine and spermidine were added in the culture medium of *E. coli* MA255 at 0.05 mM (Theodorou et al., 2006; Filippou et al., 2007).

HI (Serva, Maryland, USA), at final concentrations of 0.5–5 mM or 0.05–50 μg/ml C48/80 (Sigma Chem. Co, MO, USA) were added in the cultures when A_{600} reached 0.2–0.3. Control cultures in the absence of any agent were included in all experiments.

β-Galactosidase assay

β-Galactosidase activity assays were performed following addition of HI and/or C48/80 using the *E. coli* MA255 (*speC*$^-$, *speB*$^-$) and the isogenic BW25113 (*atoSC*$^+$) and BW28878(Δ*atoSC*) strains, carrying plasmid pCPG5 or pCPG6 (Fig. 1), as described previously (Miller, 1992).

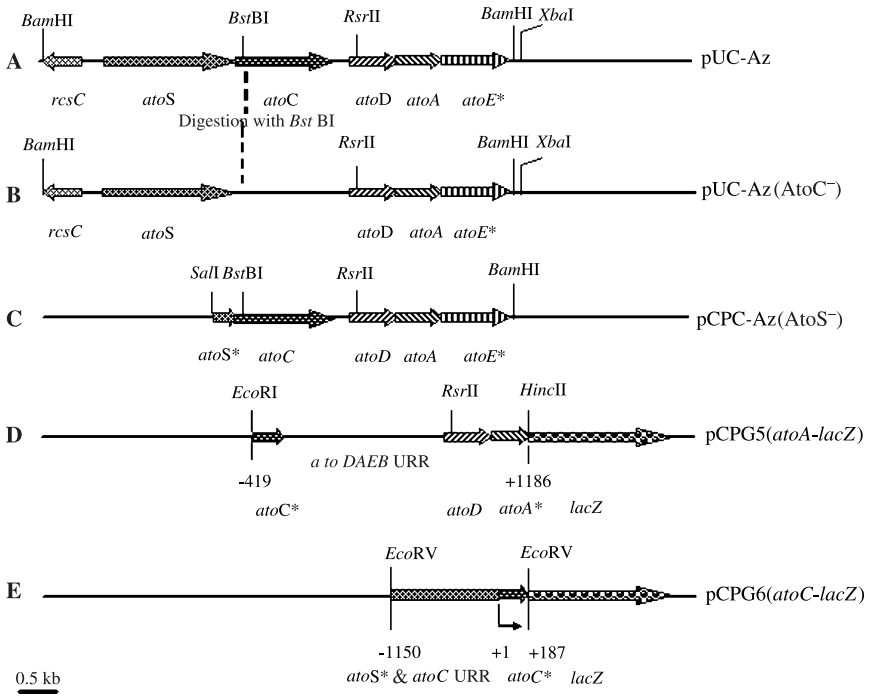

Fig. 1. Maps of the plasmids used in this study. * Denotes part of the gene

Electrophoresis and immunoblotting

Culture aliquots of 1 ml were subjected to SDS-polyacrylamide gel electrophoresis using 10% (w/v) polyacrylamide gels containing 0.1% (w/v) SDS. Gels were stained with either Coomassie Brilliant Blue R250 or silver nitrate (Miller, 1992) and proteins were transferred to nitrocellulose membranes and immunostained with rabbit polyclonal anti-AtoC, anti-AtoS (Lioliou et al., 2005) and anti-ODC (Panagiotidis et al., 1994) antibodies.

Determination of cPHB

Culture samples were collected at specified time points and processed for crotonic acid/cPHB determination. The quantification of cPHB (Theodorou et al., 2006) was based on the acid-catalyzed β-elimination of cPHB to crotonic acid, which was quantified by HPLC on an Aminex HPX-87H ion exclusion organic acid analysis column (Bio-Rad) using a Shimadzu (Tokyo, Japan) HPLC chromatography system. The cPHB content was determined from the amount of crotonic acid using the established conversion rate (Tagushi et al., 2001).

Results

Effect of HI and C48/80 on the transcription of the atoC and atoDAEB operon genes

In *E. coli* MA255 mutant strain lacking the genes expressing ODC (*speC*$^-$) and agmatine ureohydrolase (*speB*$^-$), HI induced the expression of *atoC*, but did it not activate the *atoDAEB* operon promoter (Fig. 2). Regarding the ability of the reporter constructs to respond to HI in the isogenic

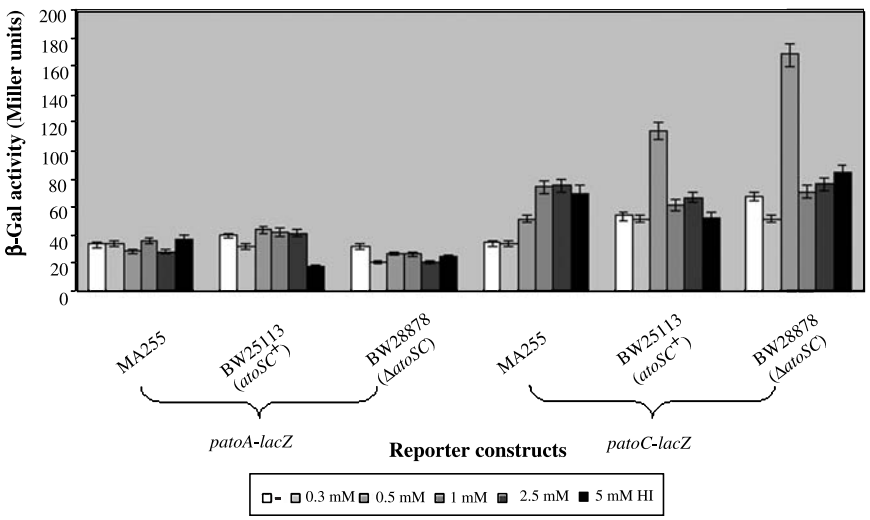

Fig. 2. Effect of HI on the transcription of the *atoC* gene and the *atoDAEB* operon. Activation of *atoC* and *atoDAEB* promoters in *E. coli* MA255 (*speB*$^-$, *speC*$^-$), BW25113 (*atoSC*$^+$) and BW28878 (Δ*atoSC*) transformed with plasmids pCPG5 (*patoA-lacZ*) or pCPG6 (*patoC-lacZ*). β-Galactosidase activity was expressed in Miller units

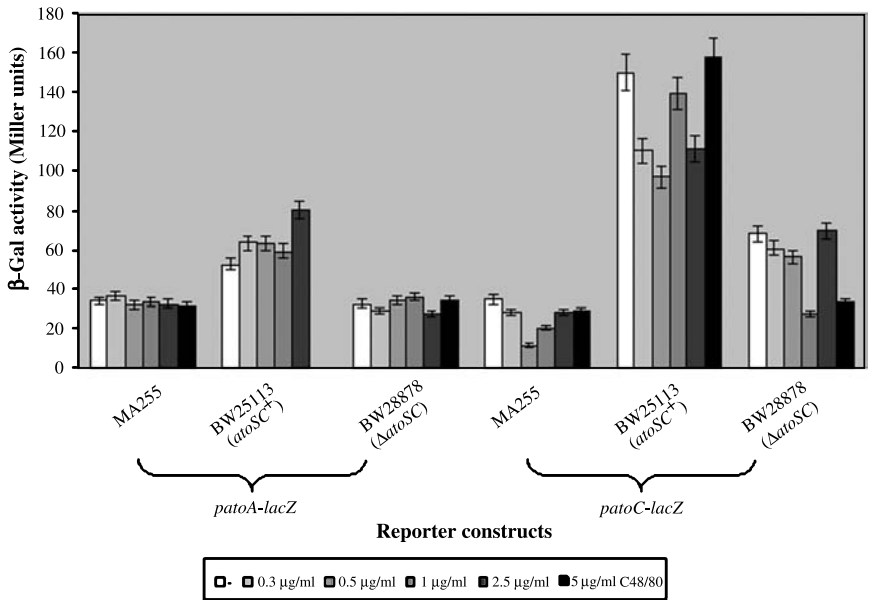

Fig. 3. Effect of C48/80 on the transcription of the *atoC* gene and the *atoDAEB* operon. Activation of *atoC* and *atoDAEB* promoters in *E. coli* MA255 (*speB*$^-$, *speC*$^-$), BW25113 (*atoSC*$^+$) and BW28878 (Δ*atoSC*) transformed with plasmids pCPG5 (*patoA-lacZ*) or pCPG6 (*patoC-lacZ*). β-Galactosidase activity was expressed in Miller units

E. coli strains BW25113 (*atoSC*$^+$) and BW28878 (Δ*atoSC*), a significant increase in *atoC* expression was observed with 0.5 mM HI, yet no detectable modification was observed at any other concentration of the amine (Fig. 2).

On the other hand, the synthetic polyamine C48/80 showed a tendency to suppress *atoC* expression but it was unable to induce any significant alteration to *atoDAEB* operon expression (Fig. 3).

The expression of AtoC and ODC proteins upon incubation with either HI or C48/80 was confirmed by immunoassay in all cases (data not shown).

Modulation of cPHB biosynthesis by HI and C48/80 in E. coli overproducing AtoS and AtoC

In *E. coli* BW25113 containing the pUC-Az plasmid, HI addition did not affect growth (Fig. 4A), while the shift to the right of the growth curve upon addition of C48/80 was indicative of an increase in the generation time (Fig. 4B). In these cells containing multiple copies of the AtoS–AtoC TCS, HI reduced cPHB levels (Fig. 5), while incubation with 1 μg/ml C48/80 resulted in increases in cPHB levels (Fig. 5). Moreover, a 10-fold increase in C48/80 concentration shifted the maximal induction of cPHB biosynthesis at 8 h of growth (Fig. 5).

Effect of HI and C48/80 on cPHB biosynthesis in E. coli possessing a functional AtoS–AtoC TCS

The deletion of the *AtoS–AtoC* genetic locus in Δ*atoSC* cells resulted in decreased basal cPHB levels compared to their *atoSC*$^+$ counterparts (Fig. 6A). Further reduction of

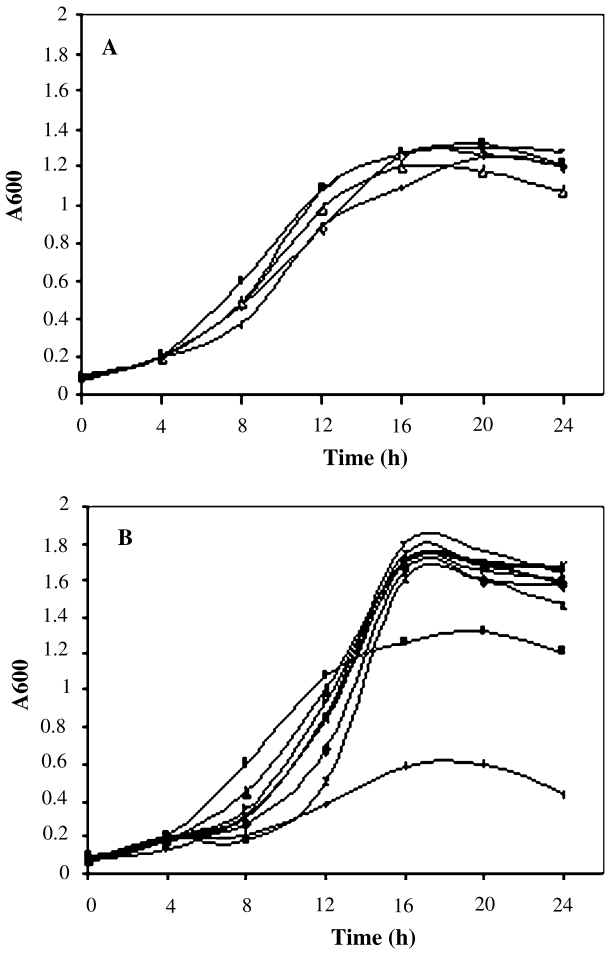

Fig. 4. Growth curve of *E. coli* BW25113/pUC-Az in the presence of (A) HI or (B) C48/80. (■) Control; presence of (◇) 0.25, (−) 1, (△) 2 or (+) 5 mM HI and (●) 0.05, (▲) 0.1, (−) 0.5, (◆) 1.0, (×) 2.5, (★) 5, (–) 7.5, (∗) 10 or (+) 50 μg/ml C48/80

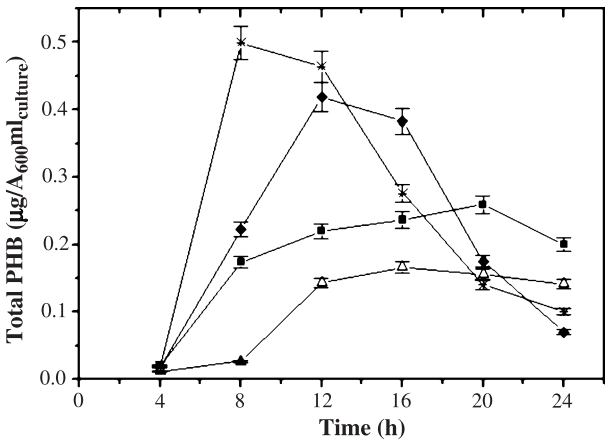

Fig. 5. Effect of HI and C48/80 on cPHB biosynthesis in *E. coli* BW25113/pUC-Az cells overexpressing the AtoS–AtoC TCS. cPHB biosynthesis in (■) control cultures and following addition of (△) 2 mM HI, (◆) 1 μg/ml C48/80 or (∗) 10 μg/ml C48/80

cPHB biosynthesis was observed following incubation with 2 mM HI (Fig. 6A). Moreover, upon HI addition, the extrachromosomal introduction of multiple copies of the AtoS–AtoC TCS by the pUC-Az plasmid in Δ*atoSC* cells increased cPHB biosynthesis to levels similar to those observed in the untransformed *atoSC*$^+$ parent strain (Fig. 6B). Finally, upon introduction of *atoC* or *atoS* alone in Δ*atoSC* cells, cPHB biosynthesis in the presence of HI remained at lower levels compared to the pUC-Az-transformed cells (Fig. 6C).

On the other hand, 1 μg/ml C48/80 caused a slight enhancement of cPHB amounts in *atoSC*$^+$ cells (Fig. 7A). C48/80-mediated enhancement of cPHB biosynthesis was more pronounced in *atoSC*$^+$ cells transformed with pUC-Az plasmid which overexpressed both components of the TCS (Fig. 7B). In Δ*atoSC E. coli*, although the compound was not able to complement the Δ*atoSC* phenotype and retained the biopolymer amounts in even lower levels (Fig. 7A), a C48/80-mediated induction was observed in BW28878/pUC-Az cells (Fig. 7B). This phenotype was similar to cPHB levels observed in *atoSC*$^+$ cells, expressing the endogenous copies of AtoS–AtoC TCS (Fig. 7A). Furthermore, in pUC-Az(AtoC$^-$) or pCPC-Az(AtoS$^-$)-transformed Δ*atoSC* cells cPHB biosynthesis in the presence of C48/80 increased compared to untransformed cells, yet they failed to up-regulate the biopolymer biosynthesis to the levels of AtoS–AtoC expressing cells (Fig. 7C).

HI counteracts the C48/80 effect on cPHB biosynthesis

In pUC-Az-transformed BW25113 cells, overexpressing the AtoS–AtoC TCS, addition of 2 mM HI at 4, 8 or 4 and

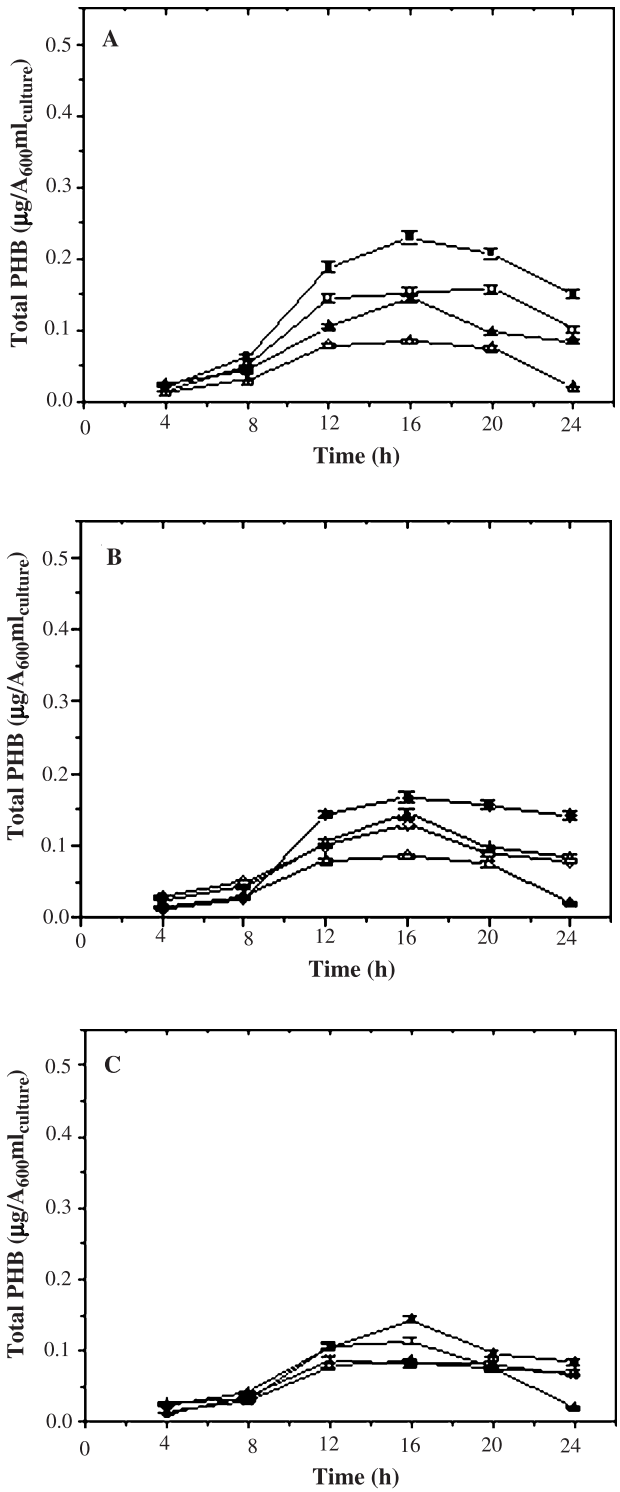

Fig. 6. The involvement of HI in cPHB biosynthesis regulation by the AtoS–AtoC TCS. BW25113 (*atoSC*$^+$) (filled symbols) and BW28878 (Δ*atoSC*) (open symbols); (A) control (■, □) or presence (▲, △) of 2 mM HI; (B) untransformed (▲, △) or transformed with plasmid pUC-Az (◆, ◇) cells grown in the presence of 2 mM HI; (C) untransformed BW28878 (Δ*atoSC*) (△) and BW25113 (*atoSC*$^+$) (▲) cells, BW28878 (Δ*atoSC*) cells transformed with pCPC-Az (|) or pUC-Az(AtoC$^-$) (∗) grown in the presence of 2 mM HI

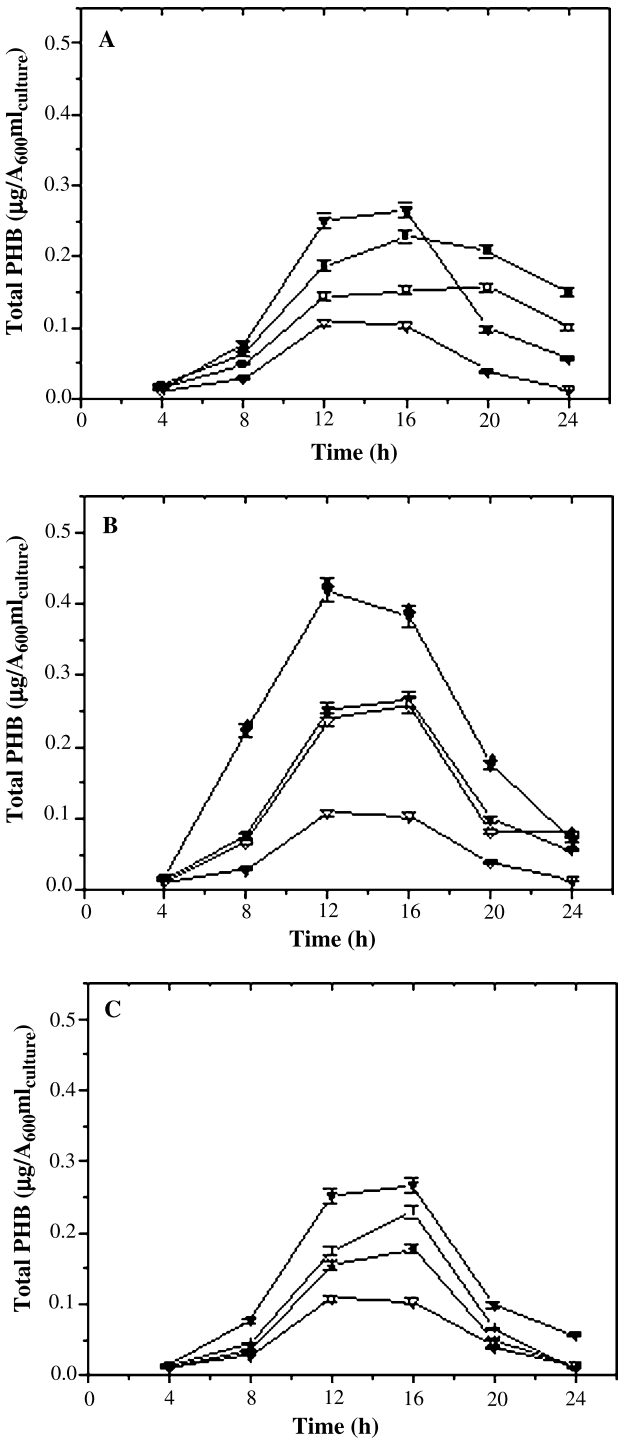

Fig. 7. The involvement of C48/80 in the cPHB biosynthesis regulation by the AtoS–AtoC TCS. BW25113 (*atoSC*$^+$) (filled symbols) and BW28878 (Δ*atoSC*) cells (open symbols); (A) control (■, □) or presence (▼, ▽) of 1 μg/ml C48/80; (B) untransformed (▼, ▽) or transformed with plasmid pUC-Az (◆, ◇) cells grown in the presence of 1 μg/ml C48/80; (C) untransformed BW28878 (Δ*atoSC*) (▽) and BW25113 (*atoSC*$^+$) (▼) cells, BW28878 (Δ*atoSC*) cells transformed with pCPC-Az (|) or pUC-Az(AtoC$^-$) (∗) grown in the presence of 1 μg/ml C48/80

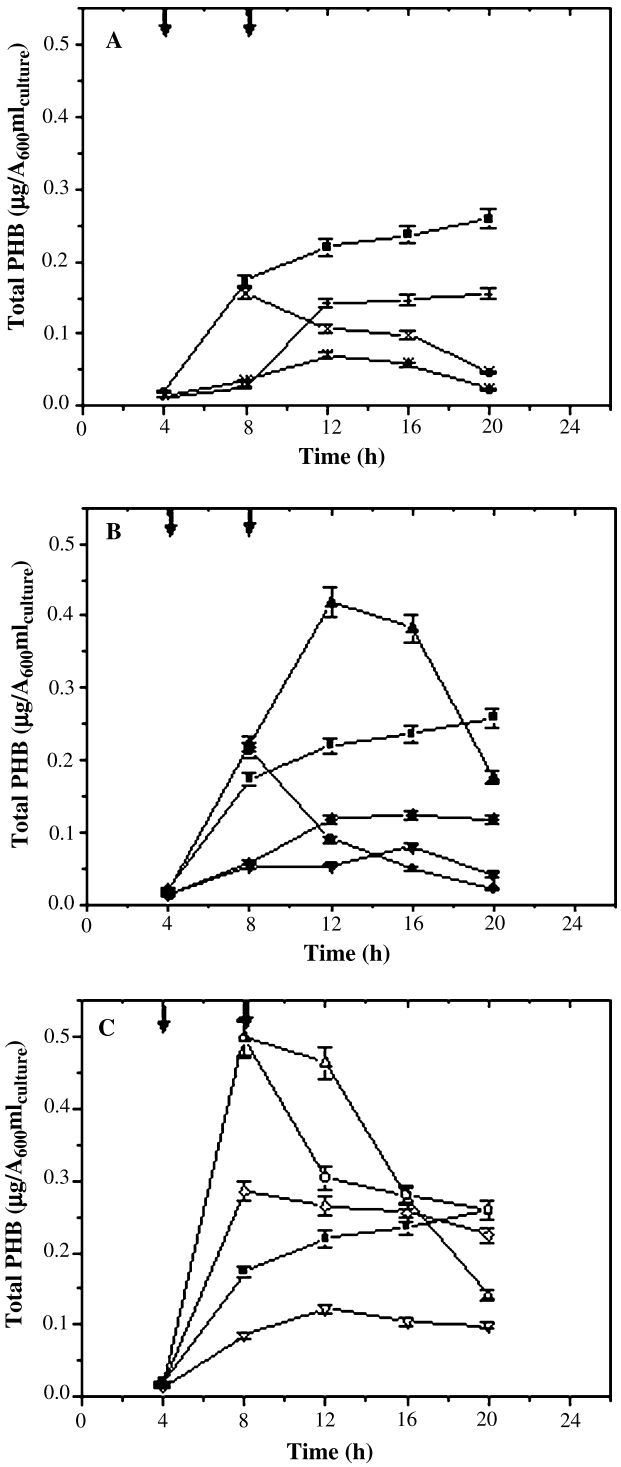

Fig. 8. Counteraction of the C48/80 effect on cPHB biosynthesis by HI in BW25113/pUC-Az cells. (A) HI alone was added at (+) 4 h, (x) 8 h or (∗) 4 and 8 h; cells were grown in the presence of (B) 1 μg/ml or (C) 10 μg/ml C48/80. HI, at 2 mM HI, was added at 4 h and/or 8 h of growth as indicated by the arrows. (■) control; (▲, △) C48/80, (◆, ◇) C48/80 plus HI at 4 h, (●, ○) C48/80 plus HI at 8 h, (▼, ▽) C48/80 plus HI at 4 and 8 h

8 h of growth resulted in reduced cPHB biosynthesis (Fig. 8A). The inductive effect of 1 μg/ml C48/80 on cPHB biosynthesis was counteracted by the co-administration of HI at any time point (Fig. 8B), the respective cPHB levels being comparable to those observed upon exposure to HI alone (Fig. 8A). Increased concentrations of 10 μg/ml C48/80 shifted the maximal increase of cPHB levels from 12 to 8 h of growth (Fig. 8C). Co-administration of HI at 4 or 8 h of growth partially antagonized the effect of this increased concentration of C48/80. The amine circumvented the action of C48/80 on cPHB biosynthesis when added at both 4 and 8 h of growth (Fig. 8C).

Discussion

The contribution of TCSs in bacterial pathogenicity (Guarner and Malagelada, 2003) in combination to the multiple activities of HI in the organism (Novak and Falus, 1997; Crosa and Walsh, 2002; Hori et al., 2002; Akdis and Blaser, 2003; Guarner and Malagelada, 2003; Kakavas et al., 2006; Jules and Buchrieser, 2007) led this investigation of the possible involvement of HI and C48/80 on the AtoS–AtoC signal transduction pathway.

The effects of HI and C48/80 on *atoC* but not on *atoDAEB* operon transcription was indicative of their selectivity, in accordance to the recently reported differential modulation of *atoC* transcription by structurally and functionally related diamines (Filippou et al., 2007). In contrast to the natural polyamines, the non-natural diaminopropane has been reported to activate *atoC* transcription in vivo and this activity was suggested to depend on the distance between the two amino groups (Filippou et al., 2007).

The indication that HI and C48/80 may elicit intracellular actions through the AtoS–AtoC TCS pointed to the investigation of the possible association of these agents with cPHB biosynthesis, a downstream target of the AtoS–AtoC TCS (Theodorou et al., 2006, 2007). HI elicited an inhibitory effect on cPHB biosynthesis, irrespective of the expression or the number of AtoS–AtoC TCS copies expressed in *E. coli* (Fig. 6). On the other hand, C48/80 induced cPHB biosynthesis in *E. coli*, maximal induction being observed in cultures overexpressing both components of the AtoS–AtoC TCS (Fig. 7).

Interestingly, HI counteracted the actions of C48/80 on cPHB biosynthesis, upon co-administration of the agents, irrespective of the time of amine addition during bacterial growth (Fig. 8). A competition between the AtoS–AtoC inducers acetoacetate and spermidine has been reported previously (Theodorou et al., 2007). However, despite the fact that cPHB biosynthesis induction has been typically attributed to the *atoDAEB* transcriptional activation (Theodorou et al., 2006) the mechanisms underlying the circumvention of C48/80-mediated cPHB induction by HI are far from clear.

It is worth noting that the effects of HI and C48/80 on cPHB biosynthesis (Figs. 6, 7) were opposite to their respective effects on *atoC* transcription (Figs. 2, 3). Thus, the selective HI-induced activation of *atoC* but not of *atoDAEB* operon, in combination with the inhibitory effect on cPHB biosynthesis in *E. coli* points to multiple effects of this biologically important amine (Lioliou and Kyriakidis, 2004) in a symbiotic microorganism of the GI flora (Guarner and Malagelada, 2003). Likewise, the C48/80-mediated effects on *atoC* transcription and the requirement of both TCS components for optimal cPHB up-regulation in the presence of the polyamine argue for additional mechanisms underlying the actions of the two agents in AtoS–AtoC signaling (Lioliou and Kyriakidis, 2004). Given the physiological and pathophysiological significance of HI (Novak and Falus, 1997; Hori et al., 2002), the involvement of the RR of the AtoS–AtoC TCS or ODC in a modulatory signal mediated by the biogenic amine deserves careful consideration, without excluding at present the contribution of additional regulatory pathways. Along this line of research, polycationic agents, like C48/80, have been shown to disrupt membrane permeability in *E. coli* and to increase Ca^{2+} efflux (Katsu et al., 1985).

The prominent role of HI in the host microenvironment of many bacteria, including *E. coli* provide the lead for the elucidation of yet unresolved implications of the reported interaction of amines with the AtoS–AtoC TCS in human physiology and pathophysiology.

References

Akdis CA, Blaser K (2003) Histamine in the immune regulation of allergic inflammation. J Allergy Clin Immun 112: 15–22

Blattner FR, Plunkett GIII, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y (1997) The complete genome sequence of *Escherichia coli* K-12. Science 277: 1453–1474

Canellakis ES, Paterakis AA, Huang SC, Panagiotidis CA, Kyriakidis DA (1993) Identification, cloning, and nucleotide sequencing of the ornithine decarboxylase antizyme gene of *Escherichia coli*. Proc Natl Acad Sci USA 90: 7129–7133

Crosa JH, Walsh CT (2002) Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. Microbiol Mol Biol Rev 66: 223–249

Ennis M, Atkinson G, Pearce FL (1980) Inhibition of histamine release induced by compound 48/80 and peptide 401 in the presence and

absence of calcium. Implications for the mode of action of anti-allergic compounds. Agents Actions 10: 222–228

Filippou PS, Lioliou EE, Panagiotidis CA, Athanassopoulos CM, Garnelis T, Papaioannou D, Kyriakidis DA (2007) Effect of polyamines and synthetic polyamine-analogues on the expression of antizyme (AtoC) and its regulatory genes. BMC Biochem 8: 1–15

Grigoroudis AI, Panagiotidis CA, Lioliou EE, Vlassi M, Kyriakidis DA (2007) Mutagenesis and 3D-modeling studies of the AtoS–AtoC system: altering the N-terminal AtoC domain mediates differential activities on phosphorylation, ATPase and DNA-binding in *Escherichia coli.* Biochim Biophys Acta 1770: 1248–1258

Guarner F, Malagelada JR (2003) Gut flora in health and disease. Lancet 361: 512–519

Haldimann A, Wanner BL (2001) Conditional-replication, integration, excision and retrieval plasmid-host systems for gene structure-function studies of bacteria. J Bacteriol 183: 6384–6393

Hoch JA, Silhavy TJ (eds) (1995) Two-component signal transduction. American Society for Microbiology, Washington, DC

Hori Y, Nihei Y, Kurokawa Y, Kuramasu A, Makabe-Kobayashi Y, Terui T, Doi H, Satomi S, Sakurai E, Nagy A, Watanabe T, Ohtsu H (2002) Accelerated clearance of *Escherichia coli* in experimental peritonitis of histamine-deficient mice. J Immunol 169: 1978–1983

Igarashi K, Kashiwagi K (2000) Polyamines: mysterious modulators of cellular functions. Biochem Biophys Res Commun 271: 559–564

Jenkins LS, Nunn WD (1987) Regulation of the *ato* operon by the *atoC* gene in *Escherichia coli.* J Bacteriol 169: 2096–2102

Jules M, Buchrieser C (2007) *Legionella pneumophila* adaptation to intracellular life and the host response: clues from genomics and transcriptomics. FEMS Lett 581: 2829–2838

Kakavas S, Zampeli E, Papamichael K, Delitheos B, Tiligada E (2006) The mast cell pathway to inflammation and homeostasis: pharmacological insights. Anti-Inflamm Anti-Allergy Agents Med Chem 5: 323–334

Katsu T, Shibata M, Fujita Y (1985) Dication and trication which can increase the permeability of *Escherichia coli* outer membrane. Biochim Biophys Acta 818: 61–66

Kyriakidis DA, Heller JS, Canellakis ES (1978) Modulation of ornithine decarboxylase activity in *Escherichia coli* by positive and negative effectors. Proc Natl Acad Sci USA 75: 4699–4703

Lioliou EE, Kyriakidis DA (2004) The role of bacterial antizyme: from an inhibitory protein to AtoC transcriptional regulator. Microb Cell Fact 3: 8–16

Lioliou EE, Mimitou EP, Grigoroudis AI, Panagiotidis CH, Panagiotidis CA, Kyriakidis DA (2005) Phosphorylation activity of the response regulator of the two-component signal transduction system AtoS–AtoC/Az in *E. coli*. Biochim Biophys Acta 1725: 257–268

Medina MÁ, Urdiales JL, Rodríguez-Caso C, Ramírez FJ, Sánchez-Jiménez F (2003) Biogenic amines and polyamines: similar biochemistry for different physiological missions and biomedical applications. Crit Rev Biochem Mol Biol 38: 23–59

Miller JH (1992) A short course in bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Novak I, Falus A (1997) Molecular biology and role of histamine in physiological and pathological reactions: a review. Acta Biol Hung 48: 385–394

Panagiotidis CA, Huang S-C, Canellakis ES (1994) Post-translational and transcriptional regulation of polyamine biosynthesis in *Escherichia coli*. Int J Biochem 26: 991–1001

Reush RN, Huang R, Bramble LL (1995) Poly-3-hydroxybutyrate/polyphosphate complexes form voltage-activated Ca^{2+} channels in the plasma membranes of *Escherichia coli*. Biophys J 69: 754–766

Tagushi S, Maehara A, Takase K, Nakahara M, Nakamura H, Doi Y (2001) Analysis of mutational effects of a polyhydroxybutyrate (PHB) polymerase on bacterial PHB accumulation using an in vivo assay system. FEMS Microbiol Lett 198: 65–71

Theodorou MC, Panagiotidis CA, Panagiotidis CH, Pantazaki AA, Kyriakidis DA (2006) Involvement of the AtoS–AtoC signal transduction system in poly-(R)-3-hydroxybutyrate biosynthesis in *Escherichia coli*. Biochim Biophys Acta 1760: 896–906

Theodorou MC, Theodorou EC, Panagiotidis CA, Kyriakidis DA (2007) Spermidine triggering effect to the signal transduction through the AtoS–AtoC/Az two component system in *Escherichia coli*. Biochim Biophys Acta 1770: 1104–1114

Tiligada E, Miligkos V, Delitheos A (2002) Cross-talk between cellular stress, cell cycle and anticancer agents: mechanistic aspects. Curr Med Chem – Anti-Cancer Agents 2: 553–566

Zhou L, Lei X-H, Bochner BR, Wanner BL (2003) Phenotype microarray analysis of *Escherichia coli* K-12 mutants with deletions of all two-component systems. J Bacteriol 185: 4956–4972

Authors' address: Ekaterini Tiligada, PhD (Wales), Assistant Professor of Pharmacology, Department of Pharmacology, Medical School, University of Athens, M. Asias 75, GR-115 27 Athens, Greece,
Fax: +30 2107462554, E-mail: aityliga@med.uoa.gr