Biochimica et Biophysica Acta 1780 (2008) 1023–1031

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Functional characterization of the histidine kinase of the *E. coli* two-component signal transduction system AtoS–AtoC

Panagiota S. Filippou [a], Lucy D. Kasemian [a], Christos A. Panagiotidis [b], Dimitrios A. Kyriakidis [a,c,*]

[a] *Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece*
[b] *Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece*
[c] *National Hellenic Research Foundation, 48 Vas. Constantinou Ave 11635, Athens, Greece*

ARTICLE INFO

Article history:
Received 21 December 2007
Received in revised form 5 May 2008
Accepted 5 May 2008
Available online 16 May 2008

Keywords:
AtoS
AtoC
Antizyme
atoDAEB
Two-component system
Histidine kinase
Phosphorylation

ABSTRACT

The *Escherichia coli* AtoS–AtoC two-component signal transduction system regulates the expression of the *atoDAEB* operon genes, whose products are required for short-chain fatty acid catabolism. In this study purified his-tagged wild-type and mutant AtoS proteins were used to prove that these proteins are true sensor kinases. The phosphorylated residue was identified as the histidine-398, which was located in a conserved H-box since AtoS carrying a mutation at this site failed to phosphorylate. This inability to phosphorylate was not due to gross structural alterations of AtoS since the H398L mutant retained its capability to bind ATP. Furthermore, the H398L mutant AtoS was competent to catalyze the *trans*-phosphorylation of an AtoS G-box (G565A) mutant protein which otherwise failed to autophosphorylate due to its inability to bind ATP. The formation of homodimers between the various AtoS proteins was also shown by cross-linking experiments both in vitro and in vivo.

1. Introduction

Two-component signal transduction systems (TCS) allow bacteria to adapt to changing environmental conditions by modulating the transcription of specific genes. A typical TCS consists of a sensor histidine kinase (HK), containing a conserved kinase core and a cognate response regulator (RR), containing a conserved regulatory domain [1]. Upon sensing an appropriate stimulus the dimeric sensor kinases undergo ATP-dependent autophosphorylation on a conserved histidine residue. Interaction of the phosphorylated HK with a cognate response regulator results in an RR-catalyzed transfer of the phosphoryl group to a conserved aspartate residue within the receiver domain of the RR. Such phosphorylation activates the effector domains of several bacterial RRs [2] and regulates a wide variety of processes such as chemotaxis, cell-cycle progression, virulence, sporulation, antibiotic resistance and response to nutritional stress [3,4]. The importance of TCS-mediated signal transduction processes in such a multitude of essential bacterial processes makes their components potential novel targets for pharmaceutical intervention in antimicrobial therapy [5,6].

The AtoS–AtoC TCS is one of the 29 characterized *Escherichia coli* two-component signal transduction systems [7]. AtoC has been long known to act as the positive regulator of the expression of the *atoDAEB* operon, whose genes encode enzymes involved in the catabolism of short-chain fatty acids [8–11]. In addition to its role as a transcriptional regulator, AtoC also functions as an antizyme (Az) to the key polyamine biosynthetic enzyme ornithine decarboxylase (ODC), i.e. as a polyamine-inducible, noncompetitive proteinaceous inhibitor [8,12–14]. We have shown that polyamines and synthetic polyamine analogues can induce *atoC* transcription in *E. coli* resulting in increased AtoC accumulation and subsequent inhibition of ODC activity [15].

Our group has provided biochemical evidence that AtoS is a membrane-bound sensor HK that phosphorylates the RR AtoC, constituting a TCS [16]. Using AtoS-enriched membrane preparations as kinase source, we showed that AtoC is phosphorylated both on aspartic and histidine residues. These residues were identified as the conserved aspartic-55 (D55) and the histidine-73 (H73), which is located within a characteristic "H-box". Mutation of either residue, D55G or H73L, resulted in severe reduction of AtoC phosphorylation in vitro, whereas AtoC carrying both mutations failed to phosphorylate [16]. The biological relevance of these findings was established by showing that the AtoC phosphorylation-site mutants failed to induce the *atoDAEB* promoter [16], although their DNA-binding activities were not affected [17]. AtoC is a sequence-specific DNA-binding

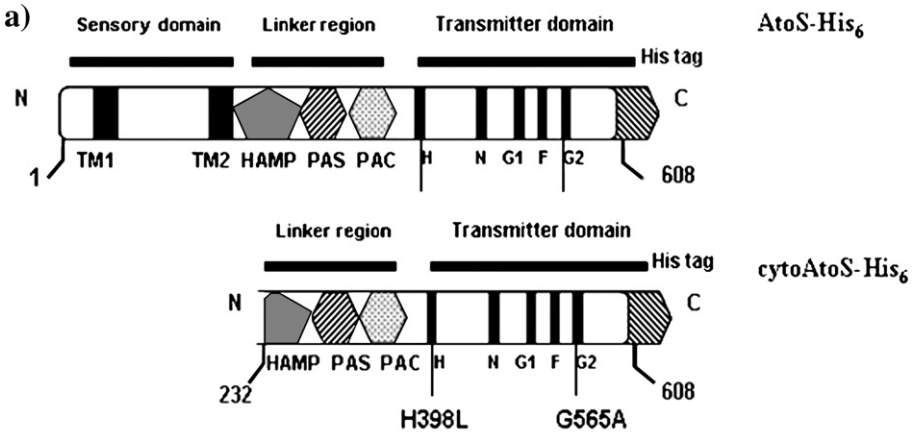
Abbreviations: Az, antizyme; ODC, ornithine decarboxylase; TCS, two-component system; HK, histidine kinase; RR, response regulator; *atoSC*, genetic locus encoding the AtoS and AtoC proteins; AcAc, acetoacetate

* Corresponding author. Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece. Tel.: +30 2310997771; fax: +30 2310997689.
E-mail address: kyr@chem.auth.gr (D.A. Kyriakidis).

0304-4165/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbagen.2008.05.002

protein that recognizes an inverted repeat consisting of two 20-bp stretches that is located upstream (−146 to −107) of the *atoDAEB* operon transcription start site. AtoC binding to this enhancer-like element (ELE) is essential for the induction of this σ54 polymerase-transcribed promoter in the presence of acetoacetate, which is the normal inducer of the AtoS–AtoC TCS [18]. In addition to its role in short-chain fatty acid catabolism the AtoSC TCS can also positively modulate the biosynthesis of poly-(*R*)-3-hydroxybutyrate (cPHB), a biopolymer with many physiological roles in *E. coli* [19]. The AtoSC TCS was also found to regulate both the biosynthesis and the intracellular distribution of cPHB in *E. coli* upon induction with spermidine, in addition to its natural inducer acetoacetate [20].

Sensor HKs are usually membrane-bound proteins consisting of an N-terminal input domain that senses and transduces the information to a C-terminal cytoplasmic transmitter domain [21]. Whilst the sensory domain is highly variable among members of the HK superfamily, the transmitter domain is rather conserved consisting of a sequence with the conserved phosphoacceptor histidine residue (H-box) and the highly conserved kinase or catalytic domain containing the other characteristic sequence "signatures" termed homology boxes, i.e. N, D, F and G-boxes [21–23]. The domain with the conserved His residue typically contains two α-helices which serve as the dimerization domain (DHp (dimerization and histidine phosphotransfer) or HisKA domain) [23,24]. The DHp or HisKA subdomain consists of two α-helices that dimerize to form a four-helix bundle, which represents the core of the transmitter domain [23,24] whereas the residues located in the N, D/F and G1 to G3-boxes participate in the formation of the nucleotide-binding cleft [21,25–26].



b)

```
AtoS  FDNTQFYSPVLDTLEHGTEHVALEISFPGRDRTIELSVTTSRIHNTHGEM 300
NtrB  ----LNIELMQESLEAGQGFTDNEVTLVIDGRSHILSVTAQRMP----DG 42
EnvZ  FR--YTLAIMLLAIGGAWLPIRIQNRPLVDLEHAALQVGKGIIP----PP 144
                                                  H-BOX
AtoS  IGALVIFSDLTARKETQRRMAQAERLATLG-ELMAGVAHEVRNPLTAIRG 349
NtrB  MILLEMAPMDNQRRLSQEQLQHAQQVAAR--DLVRGLAHEIKNPLGGLRG 90
EnvZ  LREYGASEVRSVTRAFNHMAAGVKQLADDRTLLMAGVSHDLRTPLTRIRL 194
                                                  N-BOX
AtoS  YVQILRQQTSDPIHQEYLSVVLKEIDSINKVIQQLLEFSRPRHSQWQQVS 399
NtrB  AAQLLSKALPDPSLLEYTKVIIEQADRLRNLVDRLLGPQLPGTRVTESIH 140
EnvZ  ATEMMSEQDG-----YLAESINKDIEECNAIIEQFIDYLRTGQEMPMEMA 239
                                                  N-BOX
AtoS  LNALVEETLVLVQTAGVQARVDFISELDNELSPINADRELLKQVLLNILI 449
NtrB  K---VAERVVTLVSMELPDNVRLIRDYDPSLPELAHDPDQIEQVLLNIVR 187
EnvZ  D---LNAVLGEVIAAESGYEREIETALYPGSIEVKMHPLSIKRAVANMVV 286
                                                  G1-BOX
AtoS  NAVQAISARG-------KIRIQTWQYSDSQ---QAISIEDNGCGIDLSLQ 489
NtrB  NALQALGPEGGEIILRTRTAPQLTLHGERYRLAARIDVEDNGPGIPPHLQ 237
EnvZ  NAARYG-----------NGWIKVSSGTEPNR--AWFQVEDDGPGIAPEQR 323
      F-BOX                       G2-BOX
AtoS  KRIFDPFFTTKAS----GTGLGLALSQRIINAHQGDIRVASLPGYGATFT 535
NtrB  DTLFYPMVSGREG----GTGLGLSIARNLIDQHSGKIEFTSWPGH-TEFS 282
EnvZ  KRLFQPFVRGDSARTISGTGLGLAIVQRIVDNHNGMLELGTSERGGLSIR 373

AtoS  LILPINPQGNQTV---- 548
NtrB  VYLPIRK---------- 289
EnvZ  AWLPVPVTRAQGTTKEG 390
```

Fig. 1. Domain organization of the sensor kinase AtoS from *E. coli*. a) The principal domains of the full-length and cytosolic forms of HK AtoS. The two transmembrane domains TM1 and TM2 (a.a 16–36 and a.a 190–210) the HAMP (a.a 212–259) the PAS-PAC domains (a.a 260–325 and 326–382) and the five signature conserved motifs,(named H-, N-, G1-, F-, G2) of the transmitter domain are indicated. The conserved residues mutated in this work, H398L and G565A, are also shown. b) Sequence alignment of the *E. coli* AtoS with the NtrB and EnvZ HKs. AtoS sequence alignment with the sensor NtrB, belonging to the same HK superfamily and the typical HK EnvZ, was performed by the Clustalw program. The conserved HKs motifs are boxed: H, N, G1, F, G2-boxes. Sequence identities and similarities are denoted with asterisks and dots, respectively. The following Swiss-Prot sequences were used as input; ATOS_ECOLI (608 a.a), NTRB_ECOLI (349 a.a) and EnvZ_ECOLI (450 a.a).