FEBS Open Bio 5 (2015) 209–218

FEBS openbio

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

Biochemical and spectroscopic properties of *Brucella microti* glutamate decarboxylase, a key component of the glutamate-dependent acid resistance system

Gaia Grassini [a], Eugenia Pennacchietti [a], Francesca Cappadocio [a], Alessandra Occhialini [b,c], Daniela De Biase [a,*]

[a] Istituto Pasteur-Fondazione Cenci Bolognetti, Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, 04100 Latina, Italy
[b] Université de Montpellier, Centre d'études d'agents Pathogènes et Biotechnologie pour la Santé (CPBS), F-34293 Montpellier, France
[c] CNRS, FRE 3689, CPBS, F-34293 Montpellier, France

ARTICLE INFO

Article history:
Received 21 January 2015
Revised 11 March 2015
Accepted 12 March 2015

Keywords:
Brucella microti
Glutamate decarboxylase
Cooperativity
pH-dependent activity
Chloride activation
Substituted aldamine

ABSTRACT

In orally acquired bacteria, the ability to counteract extreme acid stress (pH ⩽ 2.5) ensures survival during transit through the animal host stomach. In several neutralophilic bacteria, the glutamate-dependent acid resistance system (GDAR) is the most efficient molecular system in conferring protection from acid stress. In *Escherichia coli* its structural components are either of the two glutamate decarboxylase isoforms (GadA, GadB) and the antiporter, GadC, which imports glutamate and exports γ-aminobutyrate, the decarboxylation product. The system works by consuming protons intracellularly, as part of the decarboxylation reaction, and exporting positive charges via the antiporter.

Herein, biochemical and spectroscopic properties of GadB from *Brucella microti* (*Bm*GadB), a *Brucella* species which possesses GDAR, are described. *B. microti* belongs to a group of lately described and atypical brucellae that possess functional *gadB* and *gadC* genes, unlike the most well-known "classical" *Brucella* species, which include important human pathogens. *Bm*GadB is hexameric at acidic pH. The pH-dependent spectroscopic properties and activity profile, combined with *in silico* sequence comparison with *E. coli* GadB (*Ec*GadB), suggest that *Bm*GadB has the necessary structural requirements for the binding of activating chloride ions at acidic pH and for the closure of its active site at neutral pH. On the contrary, cellular localization analysis, corroborated by sequence inspection, suggests that *Bm*GadB does not undergo membrane recruitment at acidic pH, which was observed in *Ec*GadB. The comparison of GadB from evolutionary distant microorganisms suggests that for this enzyme to be functional in GDAR some structural features must be preserved.

© 2015 The Authors. Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

In several food-borne pathogens and orally acquired bacteria, such as *Escherichia coli*, *Shigella flexneri*, *Listeria monocytogenes* and *Lactoccoccus lactis*, the glutamate-dependent acid resistance system (GDAR) is the most efficient system in counteracting the extreme acid stress encountered by these microorganisms during their transit through the mammalian host stomach [1,2]. GDAR relies on the activities of an enzyme, glutamate decarboxylase (Gad; EC 4.1.1.15), and an antiporter (GadC) (Fig. 1). In *E. coli*, GDAR was extensively studied [1]. Following a drop of the extracellular pH to 2.5 or lower, (i) the cytoplasm becomes acidic (pH ⩽ 5.0) because the cell membrane becomes leaky to protons (H^+); (ii) the intracellular acidification activates Gad, which at each catalytic cycle consumes an intracellular H^+ while converting L-glutamate (L-Glu) into γ-aminobutyrate (GABA); (iii) the proton-consuming activity of Gad is coupled to the electrogenic antiport carried out at this same pH values by GadC, which provides to the L-Glu^0/$GABA^{+1}$ antiport [3,4]. Thus, by consuming H^+ and exporting positive charges, the decarboxylase and the antiporter make up an efficient molecular system which protects the bacteria

Abbreviations: Abs, absorbance; GABA, γ-aminobutyrate; GadB, glutamate decarboxylase (B isoform); *Bm*GadB, *Brucella microti* GadB; *Ec*GadB, *Escherichia coli* GadB; GDAR, glutamate-dependent acid resistance; PLP, pyridoxal 5′-phosphate

* Corresponding author at: Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Corso della Repubblica 79, 04100 Latina, Italy. Tel.: +39 0773 1757212.

E-mail address: daniela.debiase@uniroma1.it (D. De Biase).

http://dx.doi.org/10.1016/j.fob.2015.03.006
2211-5463/© 2015 The Authors. Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

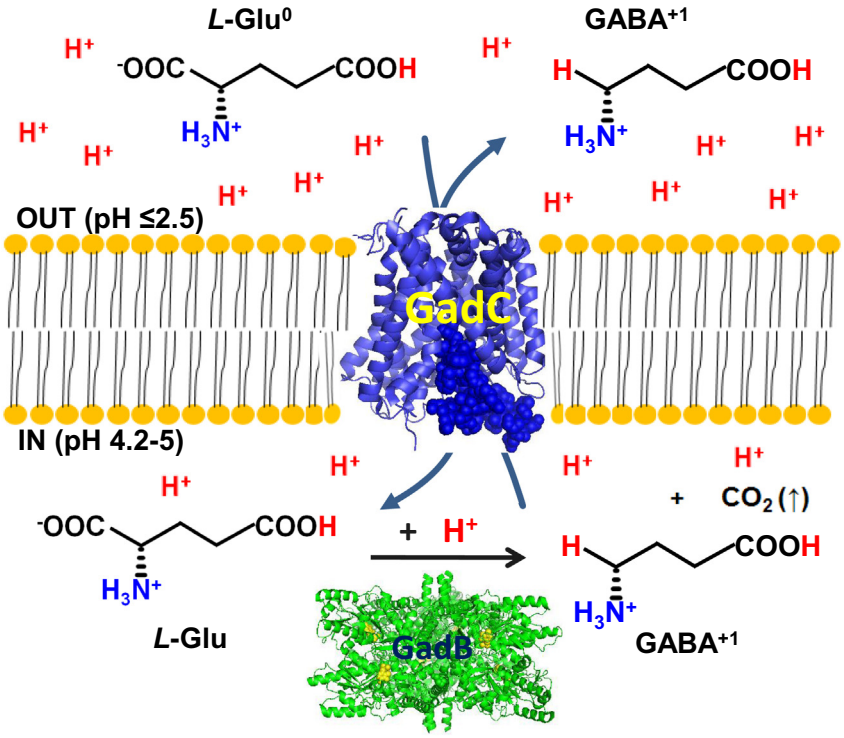

Fig. 1. Schematic representation of the role played by the major structural components of the *E. coli* GDAR system, the most extensively investigated AR system [1]. L-glutamate (L-Glu, net charge 0) is taken up by the electrogenic L-Glu^0/GABA^{+1} antiporter GadC, an inner membrane protein. Decarboxylation of L-Glu via GadA/B consumes an intracellular H^+ at each catalytic cycle, while GadC contributes to the generation of proton motive force by GABA (net charge +1) export. The structures of GadB at acidic pH (PDB: 1PMM) and GadC at pH 8.0 (PDB: 4DJK), the only one currently available, are shown as ribbon drawing generated with PyMol. The C-plug that in GadC is locking the substrate entry channel is shown in filled space.

from a life-threatening acidification of the cytoplasm. The structural bases underlying GadB and GadC activities in *E. coli* have been unveiled and share striking similarities [5,6]. Both proteins undergo auto-inhibition at pH >5.5: GadB uses a 15 amino acid-long C-terminal tail to plug the access to the active site [5], whereas GadC engages the last 41 amino acids residues in its sequence, localized on the cytoplasmic side of the inner membrane, to lock the substrate entry channel [6] (Fig. 1). Notably, in both proteins these structural elements contain histidine residues playing a crucial role as gate-keepers.

While *E. coli* possesses one copy of the GadC coding gene (*gadC*) and two genes (*gadA* and *gadB*) coding for the glutamate decarboxylase isoforms, GadA and GadB, many bacteria possess only one decarboxylase-coding gene (*gadB*) and one antiporter-coding gene (*gadC*), nonetheless they still display GDAR [1]. Amongst them, there is *Brucella microti* CCM4915, an environment-borne pathogenic *Brucella* species isolated from common vole [7], red fox [8] and soil [9], as well as several recently described, atypical *Brucella* species (*Brucella inopinata* BO1 and *B. inopinata*-like BO2 isolated from humans, *Brucella* spp. from African frogs) [10–12] and brucellae isolated from marine mammals (*Brucella ceti* and *Brucella pinnipedialis*).

The *gadB* and *gadC* genes of *B. microti* were recently shown to participate in GDAR [13]: they play an essential role in GDAR *in vitro* and contribute to the survival of *B. microti* in a murine model of infection following oral inoculation. More recently, also the lately described brucellae and those from marine mammals were shown to possess GDAR, unlike the most well-known "classical" terrestrial *Brucella* species pathogenic for livestock, domesticated animals and man (i.e. *Brucella melitensis*, *Brucella abortus*, *Brucella suis*, *Brucella canis* and *Brucella ovis*) [14]. In the "classical" terrestrial species *gadB* and/or *gadC* genes are in fact inactivated by stop codons and/or frameshift mutations [15] and therefore the GDAR system was found to be not functional [14]. The reason for these genotypic differences is still unclear, though it might be related to a specific adaptation of each *Brucella* strain/species to a different environment and to stresses encountered during its lifecycle [14]. This is clearly an interesting aspect if one considers that the World Health Organization has estimated brucellosis as the most widespread bacterial zoonosis [16].

Prompted by (i) the finding that in *B. microti* and many other *Brucella* species GadB participates in GDAR [13,14] and (ii) the new knowledge on the mechanisms controlling the intracellular activity and cellular localization of *Ec*GadB [5,17,18], a detailed biochemical characterization of *B. microti* GadB (*Bm*GadB) was undertaken. *Bm*GadB shares with *E. coli* GadB (*Ec*GadB) 73% sequence identity, including a set of strictly conserved residues which are known to occupy critical positions, i.e. in the active site or at sites where pH-dependent conformational changes occur in *Ec*GadB [1]. In the last decade it was shown that these conformational changes in *Ec*GadB can be clearly monitored spectroscopically and by cellular localization analysis of the protein [5,17,18], thereby avoiding the need to set up more laborious crystallographic investigations. Therefore in this work the kinetic and spectroscopic properties of recombinant *Bm*GadB were compared with those of the thoroughly characterized *Ec*GadB. Overall, our study demonstrates that GadBs from evolutionary distant microorganisms, such as *E. coli* (a Gammaproteobacterium) and *B. microti* (an Alphaprotebacterium), share many biochemical properties and structural features which are instrumental for the full development of GDAR.

Download English Version:

https://daneshyari.com/en/article/1981577

Download Persian Version:

https://daneshyari.com/article/1981577

Daneshyari.com