Review

Chaperone-dependent mechanisms for acid resistance in enteric bacteria

Weizhe Hong[1,2*], Ye E. Wu[1,2*], Xinmiao Fu[1] and Zengyi Chang[1,3]

[1] State Key Laboratory of Protein and Plant Gene Research, School of Life Sciences, and Center for Protein Science, Peking University, Beijing, 100871, China
[2] Department of Biology, Stanford University, Stanford, CA 94305, USA
[3] Center for History and Philosophy of Science, Peking University, Beijing 100871, China

The extremely acidic environment of the mammalian stomach not only serves to facilitate food digestion but also acts as a natural barrier against infections of food-borne pathogens. Many pathogenic bacteria, such as enterohemorrhagic *Escherichia coli*, can breach this host defense and cause severe diseases. These pathogens have evolved multiple intricate strategies to overcome the bactericidal activity of acids. In particular, recent studies have uncovered the central roles of two periplasmic chaperones, HdeA and HdeB, in protecting enteric bacteria from extremely acidic conditions. Here, we review recent advances in the understanding of the acid resistance mechanisms of Gram-negative bacteria and focus on the mechanisms of HdeA and HdeB in preventing acid-induced protein aggregation and facilitating protein refolding following pH neutralization.

Acid resistance in host–pathogen interactions

The mammalian stomach normally contains an extremely acidic environment which acts as a natural barrier against infection by food-borne pathogens [1]. Although this acidic environment has a pH value as low as 1–3, many bacterial pathogens, such as *Escherichia coli* and *Helicobacter pylori*, survive this host defense and cause diseases [2–5]. *H. pylori* is the strongest known risk factor for gastroduodenal ulcers and is classified as a carcinogen for gastric cancers [5]; enterohemorrhagic *E. coli* causes infections associated with hemolytic uremic syndrome and may lead to death [4]. How these bacterial pathogens overcome an acidic environment has been a fundamental question in understanding host–pathogen interactions.

In acidic environments, protons (H^+) may leak into the cell and markedly reduce the cytoplasmic pH [6]. The detrimental effects of acidic pH, including protein unfolding and aggregation [7,8], lead to disruption of normal biological processes and damage to cellular structures, eventually causing cell death [1,3]. Thus, to survive in such acidic conditions, bacterial pathogens need to develop mechanisms to prevent the acidification of their intracellular environment and alleviate potential acid-induced damage. Accumulating evidence suggests that different pathogens adopt different strategies to achieve this goal.

H. pylori, a human gastric pathogen, has developed multiple mechanisms to colonize near the surface of the human gastric mucosa [5]. *H. pylori* generates large quantities of urease, which consumes a proton to convert ammonia to ammonium, and eventually neutralizes the cytoplasmic pH. In addition, urease-independent mechanisms have been found for adaptation to mildly acidic conditions [9]. However, even with protection by multiple acid resistance mechanisms, *H. pylori* is still vulnerable to acid and only survives for a few minutes at a low pH. To achieve long-term survival in the stomach, *H. pylori* senses the pH gradient within the mucus layer and swims towards a neutral pH environment at the mucous epithelial surface by chemotaxis [10–14]. This strategy helps *H. pylori* stay away from harsh gastric acid.

By contrast, the human enteric pathogen enterohemorrhagic *E. coli*, as well as many other enteric bacteria, normally colonizes the intestine and utilizes different strategies to survive gastric acid as it travels through the stomach (Figure 1). These enteric bacteria and a few other related microbes prefer to live at neutral pH conditions but are capable of surviving extremely acidic environments for at least 2 h (Table 1) [15]. Not surprisingly, the level of acid resistance in each microbe is inversely correlated with the dose needed to cause infections (Table 1). For example, *E. coli* and *Shigella flexneri*, which are the most acid resistant, have lower infectious doses, whereas *Vibrio cholerae*, which is less acid resistant, has a higher infectious dose. In this review, we discuss different strategies Gram-negative enteric bacteria adopt to prevent acidification of their intracellular environment and alleviate potential acid-induced damage.

Acid resistance mechanisms in enteric bacteria

To survive in an extremely acidic environment, many enteric bacteria employ amino acid decarboxylase systems that decarboxylate glutamate, arginine or lysine (Table 1, Figure 1) [15,16]. In each of these systems, a cytoplasmic decarboxylase converts its substrate into a respective amine (γ-aminobutyric acid, agmatine or cadaverine) and an antiporter exchanges the imported amino acid for the cytoplasmic amine produced [17–20]. These systems consume one cytoplasmic proton during amino acid decarboxylation, which in turn raises the cytoplasmic pH [6]. Therefore, while protons continuously leak into the cytoplasm, the cytoplasmic pH could be maintained at ~pH 4.5, a level that could be tolerated by the bacteria [6].

Corresponding author: Chang, Z. (changzy@pku.edu.cn)
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* These authors contributed equally to this manuscript.

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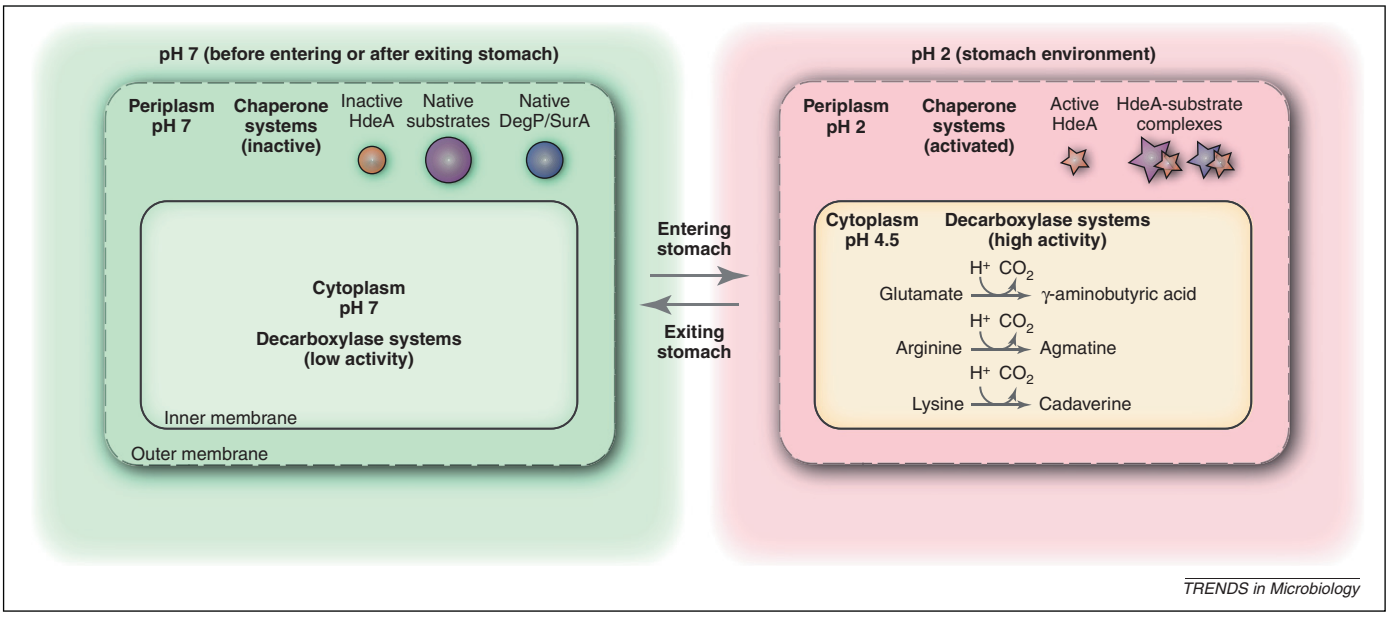

Figure 1. Acid resistance mechanisms in Gram-negative enteric bacteria. This schematic shows enteric bacteria traveling from a neutral pH environment (left panel) to an acidic environment in the stomach (right panel). The cytoplasm is enclosed by the inner membrane with low permeability (solid line) and the periplasm is enveloped by the outer membrane with high permeability (dashed line). When the bacteria enter the stomach, the pH in the periplasm decreases rapidly to the same level as the environmental pH (~pH 2) due to the permeability of the outer membrane to protons. In the periplasm, the chaperone HdeA (and HdeB) represents the major protection mechanism. HdeA is inactive and does not bind to native substrate proteins at a neutral pH (left panel). Upon the pH decrease, HdeA is activated and binds to misfolded substrate proteins to prevent their aggregation (right panel). Although protons may leak into the cytoplasm, the cytoplasmic pH could be maintained at ~pH 4.5, which is mainly attributed to three amino acid decarboxylase systems that efficiently consume protons via decarboxylation. Different decarboxylate systems have different optimum pH values, enabling them to provide protection over a certain pH range (right panel). Round balls represent proteins in a well-folded conformation and stars represent proteins in an unfolded conformation.

Interestingly, different decarboxylase systems have different optimum pH values, which are correlated with the environmental pH that the bacteria can tolerate (Table 1). The glutamate decarboxylase system with a lower pH optima of pH ~4 supports the bacteria in a lower pH environment (pH 2). *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and *V. cholerae*, which do not possess this system, are sensitive to an acidic environment at pH 2. Arginine and lysine decarboxylase systems are also significant, as they have higher pH optima (pH 5–6) and may support survival in less acidic environments. *S. flexneri*, which does not possess these systems, survives poorly in an acidic environment with a relatively higher pH (pH 3–4.5).

Furthermore, bacteria can reverse their cytoplasmic membrane potential, which slows the influx of protons into the cell [6]. This strategy is also efficiently employed by acidophiles, microorganisms that normally live in highly acidic habitats (<pH 3), to deflect the inward flow of protons [21]. The decarboxylase systems and the regulation of membrane potential both appear to occur in the cytoplasm and mainly protect the cytoplasmic environment. These mechanisms have been extensively reviewed [15,22,23]. Compared with the cytoplasm, the bacterial

Table 1. Summary of common food-borne bacteria and their acid resistance systems

Bacterial species[a]	Infectious dose[b]	Survival in acid[c]		Acid resistance systems[d]				Refs
		<pH 2.5	pH 3–4.5	Glutamate decarboxylase system (pH 4)[e]	Arginine decarboxylase system (pH 5)[e]	Lysine decarboxylase system (pH 5.5–5.7)[e]	HdeA/ HdeB	
Escherichia coli	10^1–10^2	+	+	+	+	+	+	[15,16,46]
Shigella flexneri	10^1–10^2	+	–	+	–	–	+	[15,16,46,47]
Brucella abortus	10^1–10^2	+	+	+	–	–	+	[32,48–52]
Salmonella enterica serovar Typhimurium	10^3–10^9	–	+	–	+	+	–	[16,31,53,54]
Vibrio cholerae	10^6–10^{11}	–	+	–	–	+	–	[46,55–57]

[a]Three enteric bacteria (*E. coli*, *S. flexneri* and *S.* Typhimurium) as well as two related bacteria (*B. abortus* and *V. cholerae*) are listed for comparison.

[b]Infectious dose represents the number of pathogens (pathogenic subspecies) required to cause an infection in humans.

[c]Survival in acid represents the ability of the bacteria to survive in acidic environments with the indicated low pH values. '+' represents significant survival rates and '–' represents low or undetectable survival rates under the indicated pH conditions.

[d]Major acid resistance systems are listed, including three amino acid decarboxylase systems in the cytoplasm, and two chaperones, HdeA and HdeB, in the periplasm. '+' or '–' represents the presence or absence of each acid resistance system, respectively.

[e]The optimum pH for the decarboxylase in each system is indicated in parentheses.

periplasm is more vulnerable to acid-induced damage (Figure 1, see below). However, the acid resistance mechanism for the periplasmic space is considerably less well understood. In the following sections, we focus on recent advances that uncovered periplasmic mechanisms that are indispensible for supporting bacterial acid resistance.

Acid resistance mechanisms for the periplasm: *hdeA* and *hdeB*

In Gram-negative bacteria, the periplasm is enveloped by an outer membrane, which contains nonspecific transporters, such as porins, and allows free diffusion of molecules smaller than 600 Da, including protons [24,25]. When the bacteria enter the stomach, the pH in the periplasm decreases rapidly to close to the environmental pH (pH 1–3) (Figure 1). A wide range of proteins are located in the periplasm and on the inner membrane, including the enzymes and transporters essential for nutrient uptake, as well as the transmembrane antiporters involved in the decarboxylase systems [15,26]. During acid stress, these proteins could be directly exposed to a low pH environment, which is much more acidic than the cytoplasm (Figure 1). Thus, the proteins in the periplasm are more vulnerable to acid-induced damage compared with those in the cytoplasm. Indeed, both *in vitro* and *in vivo* evidence suggest that periplasmic proteins may denature and aggregate under such acidic conditions [8,27].

Recently, two periplasmic proteins, encoded by the *hdeA* and *hdeB* genes, were discovered to be key factors in supporting acid resistance in the periplasm of bacteria [8,28,29]. *hdeA* and *hdeB* are expressed under normal physiological conditions, and their expression can be further induced in response to moderately low pH [30]. Disruption of the *hdeAB* operon severely compromises the survival and growth of *S. flexneri*, *E. coli* and *Brucella abortus* under acid stress [28,29,31,32]. Reintroduction of either protein into a mutant *E. coli* strain lacking both functional *hdeA* and *hdeB* genes could partially restore survival after acid treatment, suggesting that both proteins contribute to acid resistance *in vivo* [29]. In addition, HdeA could also protect the bacteria from acid stress caused by organic acid metabolites produced during fermentation [33].

Consistent with their important roles in the physiology of acid resistance, the *hdeA* and *hdeB* genes are present in *E. coli*, *S. flexneri* and *B. abortus*, which can survive at an extremely low pH, but are absent in species that are acid sensitive below pH 3, such as *S.* Typhimurium and *V. cholerae* (Table 1) [28,31,34]. The HdeA and HdeB proteins in the periplasm and the glutamate decarboxylase system in the cytoplasm are activated at a lower pH (see below), which correlates well with the abilities of the bacteria to survive in a lower pH environment and the lower dosages needed for infection (Table 1).

A recent study further investigated the roles of HdeA and HdeB in the survival of various enterohemorrhaic *E. coli* at pH 2.0 [35]. *hdeA* and *hdeB* genes are present in many enterohemorrhaic *E. coli*, and loss of *hdeA* and *hdeB* genes caused over 100- to 1000-fold reductions in acid survival of enterohemorrhagic *E. coli* O145. In *E. coli* O157:H7 strains, however, sequence analysis revealed a mutation in the putative start codon of the *hdeB* gene, which leads to an absence of HdeB protein expression [35]. Despite the lack of HdeB, *E. coli* O157:H7 does rely on HdeA to some extent, and loss of *hdeA* gene led to a 5-fold decrease in acid survival [35].

Chaperone mechanisms for entering an acidic environment

How do HdeA and HdeB protect the bacteria from an extremely low pH? HdeA was found to prevent acid-induced aggregation of periplasmic proteins exclusively below pH 3 and was therefore regarded as a chaperone (Box 1) under acidic conditions (Figure 2a,b) [8,28]. At a neutral pH, HdeA exists as a dimer with no chaperone activity and does not bind to substrate proteins. When the pH is reduced to below 3, HdeA rapidly dissociates into monomers and exposes hydrophobic surfaces which directly bind to substrate proteins and prevent their aggregation [8,36,37].

Although HdeA has been shown to bind to several model substrate proteins *in vitro*, its *in vivo* substrates have remained largely unknown until recently. A genetically encoded protein photocrosslinking probe has been developed to examine *in vivo* protein–protein interactions in normal or acid stress conditions [38]. This approach identified a broad range of periplasmic proteins that can interact with HdeA *in vivo* at an acidic pH (Table 2); these include transport proteins, metabolic enzymes, chaperones, lipoproteins, proteases and others, many of which play important roles in various physiological processes [38]. Some of them (e.g. the chaperone SurA) were shown to be protected from acid-induced aggregation by HdeA (see below). The physiological relevance of the other identified interactions awaits further validation.

HdeB has also been shown to prevent periplasmic protein aggregation at a low pH [29]. Although *in vitro* data suggest that HdeA is more efficient than HdeB at pH 2, whereas HdeB is more efficient at pH 3, they both appear to contribute to acid resistance at pH 2 and pH 3 *in vivo* [29]. These findings raise the interesting possibility that HdeA

Box 1. Protein folding and molecular chaperones

Proper folding of proteins into correct conformations is essential for their biological functions [42]. In cells, however, newly synthesized proteins themselves may not fold efficiently. Matured proteins may also face the risk of unfolding due to stress factors. Both conditions may lead to protein misfolding and aggregation, which could be toxic to cells. To prevent such damage, a special class of proteins, namely molecular chaperones, has been evolved to promote efficient protein folding and prevent protein aggregation. A molecular chaperone is defined as any protein that interacts with and helps another protein (substrate) to acquire its functionally active conformation, without being present in its final structure [41,42]. Molecular chaperones are involved in a multitude of protein quality control processes, such as *de novo* folding, oligomeric assembly and refolding of stress-denatured proteins. Classic molecular chaperones include several classes of structurally unrelated proteins, which are usually classified according to their molecular weight (Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and small heat shock proteins). HdeA and HdeB were found to be molecular chaperones that function specifically in acidic conditions [8,28,29]. Mechanistically, molecular chaperones, including HdeA and HdeB, are believed to recognize and interact with their substrate proteins mainly through hydrophobic forces.

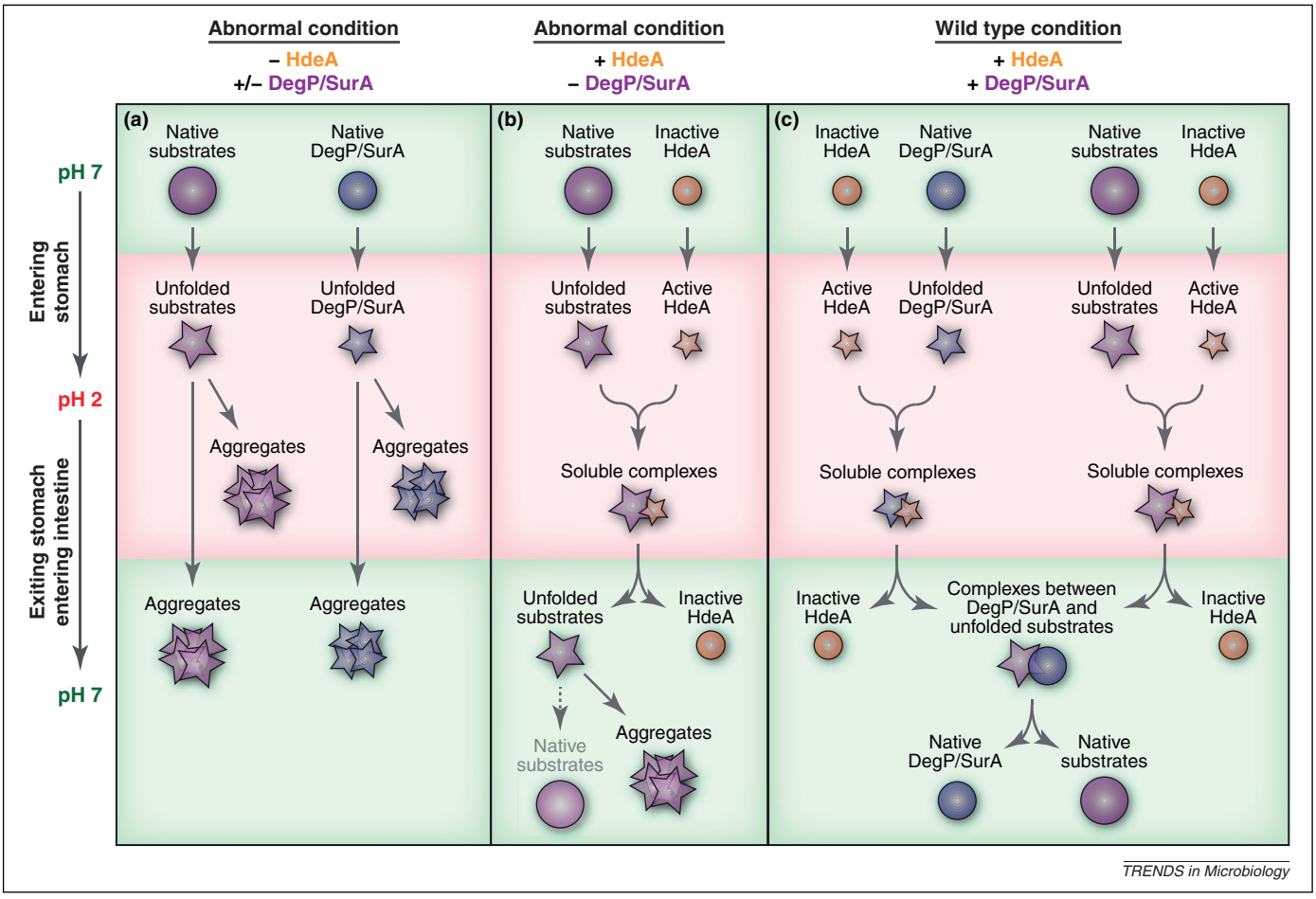

Figure 2. Model for HdeA function during and after acid stress. In a neutral environment before the bacteria enter the stomach, substrate proteins are in the native state and HdeA exists as an inactive dimer. Upon entry into the stomach, the pH decreases to below 3. **(a)** In the absence of HdeA, many substrates, including some chaperones (e.g. SurA), are misfolded and form aggregates, whereas some others (e.g. DegP) are denatured yet remain soluble, but precipitate heavily upon neutralization when the bacteria leave the stomach and enter the intestine. **(b)** When HdeA is present, it prevents acid-induced substrate aggregation by transforming into a partially disordered monomer that binds to the substrate using a hydrophobic surface and facilitates solubilization using the charged terminal regions. Upon neutralization, HdeA dissociates from the substrate and refolds into its inactive state. The substrate is slowly released from HdeA in an unfolded form, which will either refold spontaneously or aggregate subsequently. However, in the absence of other chaperones (e.g. DegP or SurA), refolding of the substrates could be limited. **(c)** HdeA can also collaborate with other chaperones, such as DegP and SurA, *in vivo*. DegP and SurA themselves are initially protected by HdeA at an acidic pH and subsequently assist in more efficient refolding of other substrate proteins upon neutralization. Round balls represent proteins in a well-folded conformation and stars represent proteins in an unfolded conformation.

and HdeB may each have a different optimum pH and may function cooperatively to support acid resistance within different pH ranges. Similar to HdeA, HdeB exists as a dimer at a neutral pH and undergoes acid-induced monomerization and conformational changes [29,39]. However, dissociation of the HdeB dimer is complete at pH 3, whereas that of HdeA is complete at a lower pH. Moreover, HdeB exposes hydrophobic surfaces to a lesser extent than HdeA does at pH 2 and pH 3. These dissimilarities might explain the differences in the chaperone activities of HdeA and HdeB at pH 2 and pH 3. Furthermore, they appear to synergistically prevent acid-induced aggregation of some model substrate proteins, although a heterodimer between HdeA and HdeB could not be detected [29]. The exact mechanism of this synergy needs further investigation.

Chaperone mechanisms for exiting an acidic environment

Many substrate proteins, although prevented from aggregation at an acidic pH by HdeA and HdeB, are nonetheless in an unfolded and inactive conformation [27,40]. Upon the exit of enteric bacteria from the acidic environment of the stomach into the neutral environment of the intestine, unfolded proteins need to be refolded to recover their activity. Remarkably, HdeA and HdeB were also found to contribute to substrate resolubilization and renaturation [27,40]. What is the mechanism underlying this function?

First, using some model substrate proteins, an *in vitro* study found that HdeA probably dissociates from the substrate upon neutralization [40]. Further analysis of substrate release kinetics has shown that the substrate is released in a slow process, which is much longer than the HdeA–substrate association process at a low pH [40]. Are substrates already refolded before being released from HdeA or released in an unfolded state? To address this question, the cytoplasmic chaperone GroEL was elegantly utilized as an *in vitro* molecular trap for non-native substrate proteins [40]. GroEL captures non-native substrates and prevents their refolding unless another chaperone, GroES, and ATP are added. The substrate reactivation upon neutralization was suppressed by the

Table 2. Summary of HdeA substrates at acidic pH [38][a]

Protein	Function
Transport proteins	
AraF	L-Arabinose transporter subunit
CstA	Peptide transporter induced by carbon starvation
DppA	Dipeptide transporter subunit
FruA	Fructose transporter subunit
GlpT	Glycerol-3-phosphate transporter subunit
MalE	Maltose transporter subunit
MetQ	D-Methionine transporter subunit
MglB	Galactose transporter subunit
OppA	Oligopeptide transporter subunit
RbsB	D-Ribose transporter subunit
TolB	Tol-Pal cell envelope complex subunit
Metabolic enzymes	
CpdB	2′,3′-Cyclic-nucleotide 2′-phosphodiesterase/3′-nucleotidase
CueO	Multicopper oxidase
FabI	Enoy acyl carrier protein reductase
FdoG	Formate dehydrogenase-O major subunit
SpeA	Biosynthetic arginine decarboxylase
Chaperones	
DegP	Serine endoprotease, required for high-temperature growth
FkpA	Peptidyl-prolyl *cis-trans* isomerase
PpiD	Peptidyl-prolyl *cis-trans* isomerase
SurA	Peptidyl-prolyl *cis-trans* isomerase
Lipoproteins	
BamC	Putative lipoprotein
Lpp	Major lipoprotein
NlpA	Inner membrane lipoprotein
Proteases	
DegQ	Serine endoprotease
YhjJ	Predicted zinc-dependent peptidase
Others	
DamX	Nonessential cell division protein
HflK	FtsH protease regulator
MdoG	Periplasmic glucan biosynthesis protein
MinD	Septum formation inhibitor
OmpA	Outer membrane nonspecific diffusion channel
OpgG	Periplasmic glucan biosynthesis protein
YaeT	Outer membrane protein assembly factor

[a]Identified HdeA substrates are categorized based on their primary biological function.

presence of GroEL, indicating the dissociated substrate is in an unfolded state [40] (Figure 2b). Therefore, the slow release of the substrate could be meaningful as it may help prevent aggregation-sensitive intermediates from suddenly accumulating to a high concentration, and thus reduce aggregation of these intermediates, providing them with a greater chance to enter the refolding pathway. Whether some degree of substrate refolding already occurs during the long release process or it only happens after dissociation of the substrate from HdeA has not yet been determined.

Second, are unfolded substrates released from HdeA capable of being refolded? When GroES and ATP were added after GroEL, they led to efficient renaturation of the released substrates [40]. This suggests that although the substrate is denatured, it appears to be in a folding-competent state that can still be recognized and handled by the GroEL/ES system. The substrate treated with acid in the absence of HdeA, however, was poorly refolded by the GroEL/ES system [40], suggesting that they form aggregates or folding-incompetent forms without HdeA. Thus, these findings support a model in which HdeA slowly releases substrates in a non-native yet folding-competent state following neutralization [40].

Finally, although HdeA has been shown to release substrates in their folding-competent forms, these dissociated substrate proteins still appeared to have relatively limited ability to further refold and recover activity on their own [8,38]. Substrate activity could be recovered to a higher level with the assistance of the GroEL/ES system *in vitro*, suggesting additional factors may further promote the HdeA-mediated renaturation of substrate proteins [40]. However, the GroEL/ES system functions in the cytoplasm in an ATP-dependent manner and is absent in the ATP-deficient periplasm [41,42]. ATP-independent periplasmic factors that facilitate this process have been a mystery. New light was shed on this issue by the recent identification of two periplasmic chaperones, DegP and SurA, as *in vivo* binding partners of HdeA at a low pH [38]. DegP and SurA are essential for quality control during the biogenesis of the outer membrane proteins [43,44]. It was shown that DegP and SurA themselves are initially protected by HdeA at a low pH and subsequently facilitate refolding of other substrate proteins during reneutralization [38] (Figure 2c). These findings reveal a novel mechanism by which different periplasmic chaperone systems function sequentially and collaboratively to assist protein refolding in an ATP-independent manner. HdeA-mediated protection of DegP and SurA represents a 'chaperone-protecting-chaperone' mechanism, which might be a robust strategy for bacteria to cope with diverse stress conditions in fluctuating environments.

In summary, these recent findings underscore the essential roles of HdeA and HdeB in supporting the survival of bacteria both during exposure to acid stresses and after they escape from acidic environments. When the bacteria encounter the acidic environment in the stomach, HdeA and HdeB help minimize the aggregation of misfolded periplasmic proteins, providing them with a greater chance to refold upon subsequent neutralization; when the bacteria later escape into the more neutral environment in the intestine, HdeA slowly dissociates from the substrate proteins, thus probably avoiding quick accumulation and aggregation of unfolded intermediates. In addition, other periplasmic chaperones (e.g. SurA and DegP) cooperate with HdeA to assist more efficient recovery of substrate activity. Together, the function of HdeA and HdeB helps maximize the survival chance of bacteria during their entry into and subsequent colonization in the host, and probably are key factors in normal and pathogenic host–microbe interactions.

Several gaps remain in our understanding of this chaperone cooperation mechanism. For DegP and SurA to efficiently assist refolding of other substrate proteins, it would seem advantageous if DegP and SurA themselves are released and refold more rapidly than other substrates. It remains unclear whether or not DegP and SurA dissociate from HdeA after neutralization. Characterizing their releasing and refolding kinetics would be of great interest. In addition, it is also unclear whether HdeA remains

associated with substrate proteins during refolding to directly assist refolding *in vivo*. Furthermore, whether DegP or SurA also associates with the HdeA–substrate complexes at low pH remains to be determined. Elucidation of these issues will provide a clearer picture for this chaperone cooperation mechanism.

Structural basis for chaperone activity

Much effort has been made to mechanistically understand the structural basis underlying the acid-induced dissociation, conformational changes and substrate-binding activities of HdeA and HdeB. The crystal structures of both HdeA and HdeB at a neutral pH have been solved [28,39,45]. Despite their low sequence identity, HdeA and HdeB share high similarities between their monomeric structures, both being compact single-domain proteins with a hydrophobic core created by four α-helices. Both HdeA and HdeB form homodimers at neutral pH, but the arrangements of the monomers in the dimers are very different; the two α2 helices are almost parallel in the HdeA dimer but almost perpendicular in the HdeB dimer [39]. Nonetheless, dimerization buries large hydrophobic surfaces in both cases. In HdeA, the dimer interface has also been shown to be utilized for substrate binding [37].

How does HdeA recognize different substrate proteins? Upon the decrease of the pH to below 3, HdeA and HdeB transition from the well-folded dimeric state to a partially disordered monomeric state [8,37,39]. In HdeA, this transition has been shown to occur very rapidly, leading to fast activation of the molecule. This feature makes HdeA well suited to protect proteins against rapid acid-induced aggregation [37]. Monomerization and partial misfolding of HdeA and HdeB lead to the exposure of internal hydrophobic regions that are proposed to bind substrate proteins [8,37]. Supporting this hypothesis, replacement of the hydrophobic residues in this region eliminated the capability of HdeA to bind substrate proteins [36,37]. In addition, by introducing a photocrosslinking probe into different sites, the binding interface between HdeA and its substrate proteins has been mapped to its hydrophobic regions [38]. However, the highly charged N- and C-termini have been shown to facilitate the solubilization of the chaperone–substrate complex [8,36]. This unique structure, which consists of a hydrophobic internal region and two highly charged terminal regions, is referred to as an amphiphilic feature [36]. This feature is conserved among HdeA homologs from several species and is essential for its function in supporting bacterial acid resistance [36].

Through intramolecular fluorescence resonance energy transfer (FRET) experiments which measure the distances between two residues in the HdeA monomer, it was elegantly shown that substrate binding induces conformational changes in the monomer. The highly plastic structure of the monomer at a low pH allows it to adopt different conformations depending on the structure of the bound substrate proteins, leading to wide substrate-binding specificity [37]. Together, these discoveries reveal an exquisite post-translational mechanism that enables a small chaperone to rapidly respond to acid stress and prevent the aggregation of a wide array of substrate proteins.

A model for the HdeA mechanism

The advances summarized above support the following intriguing model for the action mechanism of HdeA (and potentially HdeB) during acid stress and recovery in bacteria (Figure 2c). At a neutral pH, the substrate-binding activity of HdeA is constrained within a well-folded dimer in which hydrophobic surfaces involved in substrate binding are buried in the dimer interface. Upon the decrease of pH, the HdeA dimer quickly monomerizes and transforms into a partially unfolded conformation. The internal hydrophobic surfaces exposed through these changes, along with the structural plasticity of the monomer, enable HdeA to bind to a broad range of unfolded substrate proteins. Meanwhile, the charged terminal regions keep the chaperone–substrate complexes in a solubilized state. This amphiphilic feature allows HdeA to prevent the aggregation of substrate proteins or limit the size of the aggregates. When the bacteria escape from acidic conditions and re-enter more neutral environments, the reversible conformational changes of HdeA allow substrate proteins to be slowly released in a non-native but folding-competent state, effectively keeping the concentration of aggregation-prone intermediates low. The released substrate proteins are then refolded with the assistance of additional periplasmic chaperones, such as DegP and SurA, which themselves have been protected by HdeA during acid stress.

Concluding remarks

The harsh acidic environment in the mammalian stomach represents a formidable natural barrier for food-borne bacteria, including enteric bacteria that reside normally or pathogenically in the digestive tract. These bacteria have evolved elaborate acid resistance mechanisms to cope with this challenge. Recent studies regarding the periplasmic chaperones HdeA and HdeB have revealed their key functions in protecting bacteria from acid stress and greatly advanced the understanding of the mechanisms for chaperone activity at an extremely low pH. Cooperation between different chaperone systems provides more robust protection for bacteria under acid stress and could be a general strategy for bacteria to survive in diverse, fluctuating environments.

These studies also raised many open questions. One possible direction for future investigation would be to gain a more complete mechanistic understanding of the chaperone-assisted refolding process after acid stress. For example, as discussed above, several questions remain unanswered concerning how HdeA (and potentially HdeB) collaborate with other chaperones to assist substrate refolding. Another significant future direction is to further investigate the function of HdeA and HdeB *in vivo*. The newly developed photocrosslinking probe for detecting protein–protein interactions at a low pH has provided an excellent opportunity for exploring the physiological substrates of HdeA and HdeB. Although many *in vivo* binding partners have been identified for HdeA (Table 2), the physiological relevance for most of these interactions awaits further validation. For example, are these proteins indeed protected by HdeA at a low pH and is that important for acid resistance of the bacteria? In addition,

the same approach could be applied to HdeB, which will determine whether or not these two proteins have overlapping or distinct *in vivo* substrates, and whether or not HdeB also cooperates with DegP and SurA or any other chaperones. Furthermore, in-depth biophysical and biochemical studies for HdeB need to be performed, including detailed characterization of the processes of substrate binding, release and refolding, as have been done for HdeA. These joint endeavors will help create a more comprehensive picture of the acid resistance mechanisms in enteric bacteria and better understand host–microbe interactions during physiological and pathological processes.

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