HdeB Functions as an Acid-protective Chaperone in Bacteria*

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Summary: Enteric bacteria such as Escherichia coli utilize various acid response systems to counteract the acidic environment of the mammalian stomach. To protect their periplasmic proteome against rapid acid-mediated damage, bacteria contain the acid-activated periplasmic chaperones HdeA and HdeB. Activation of HdeA at pH 2 was shown to correlate with its acid-induced dissociation into partially unfolded monomers. In contrast, HdeB, which has high structural similarities to HdeA, shows negligible chaperone activity at pH 2 and only modest chaperone activity at pH 3. These results raise intriguing questions concerning the physiological role of HdeB in bacteria, its activation mechanisms, and the structural requirements for its function as a molecular chaperone. In this study, we conducted structural and biochemical studies that revealed that HdeB indeed acts as an effective molecular chaperone. However, in contrast to HdeA, whose chaperone function is optimal at pH 2, the chaperone function of HdeB is optimal at pH 4, at which HdeB is still fully dimeric and largely folded. NMR, analytical ultracentrifugation, and fluorescence studies suggest that the highly dynamic nature of HdeB at pH 4 alleviates the need for monomerization and partial unfolding. Once activated, HdeB binds various unfolding client proteins, prevents their aggregation, and supports their refolding upon subsequent neutralization. Overexpression of HdeA promotes bacterial survival at pH 2 and 3, whereas overexpression of HdeB positively affects bacterial growth at pH 4. These studies demonstrate how two structurally homologous proteins with seemingly identical in vivo functions have evolved to provide bacteria with the means for surviving a range of acidic protein-unfolding conditions.

Background: Periplasmic chaperones HdeA and HdeB are involved in the acid stress response in Escherichia coli. HdeA and HdeB use different mechanisms to prevent periplasmic protein aggregation, allowing them to function over a broad pH range. This study furthers the understanding of how enteric bacteria counteract acid stress.

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Results: Periplasmic chaperones HdeA and HdeB are involved in the acid stress response in Escherichia coli. HdeA and HdeB use different mechanisms to prevent periplasmic protein aggregation, allowing them to function over a broad pH range.

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Significance: This study furthers the understanding of how enteric bacteria counteract acid stress.

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HdeA is a well characterized chaperone that protects bacteria against low pH stress. At neutral pH, HdeA is present in its homodimeric chaperone-inactive form. Activation of HdeA occurs within seconds of the pH shift and is triggered by an abrupt drop in pH to <3. Protonation of select negatively charged residues appears to contribute to the dissociation of HdeA dimers into chaperone-active monomers concomitant with the partial unfolding of HdeA (16–18), putting HdeA into the class of conditionally disordered chaperones (19, 20). Upon pH neutralization, HdeA is capable of facilitating the refolding of its client proteins in an ATP-independent manner while returning into its dimeric chaperone-inactive conformation. A “slow-release mechanism” enables HdeA to keep the concentration of aggregation-prone substrate species at very low levels, hence facilitating client refolding (21).

Similar to HdeA, the structurally related HdeB also monomerizes and partially unfolds at pH <3 (10), yet in contrast to HdeA, HdeB displays no significant in vitro chaperone activity at pH 2 and has only modest activity when using a periplasmic extract as a substrate at pH 3 (10). The presence of HdeB does, however, promote solubilization of protein aggregates formed at pH 2 (22), implying at least some chaperone function for HdeB at low pH. Given the lack of chaperone activity of HdeB at low pH despite its high structural similarity to HdeA, we wondered about the mechanism of HdeB activation, the structural requirements for HdeB chaperone function, and the physiological role that HdeB plays in bacteria.

Here, we show that the optimal pH of HdeB is 4. Once activated as a chaperone, HdeB binds unfolded client proteins, prevents their aggregation, and facilitates their refolding upon neutralization. In contrast to HdeA, which is active when monomeric and partially unfolded, HdeB remains dimeric and apparently fully folded at pH 4. However, HdeB displays dynamic properties between pH 4 and 7, which apparently allow the chaperone to recognize and bind unfolding proteins at higher pH compared with HdeA. We present a model in which two structurally highly related proteins utilize distinct activation mechanisms that enable them to protect bacteria from the protein-unfolding effects of varying degrees of acid stress conditions.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, Media, and Growth Conditions—All strains and plasmids used in this study are listed in Table 1. For in vivo survival studies, hdeA, hdeB, or both hdeA and hdeB together were amplified from E. coli MG1655 using appropriate primers and cloned into the EcoRI and BamHI sites of plasmid pBAD18 to yield plasmids pJUD1, pJUD3, and pJUD5, respectively. Bacterial cultures were grown in LB medium supplemented with either 200 μg/ml ampicillin or 25 μg/ml kanamycin.

Protein Purification—HdeB was expressed from NEB10B cells harboring plasmid pTrc-hdeB (see Table 1) and purified following a published protocol (17). HdeB used in the NMR experiments was expressed in M9 medium supplemented with [15N]ammonium chloride (Cambridge Isotope Laboratories, Andover, MA) as sole nitrogen source. Purified and concentrated HdeB was stored in buffer A (50 mM potassium phosphate and 50 mM NaCl (pH 7.5)) and flash-frozen in liquid nitrogen. 15N-Labeled HdeB was stored in citrate buffer (pH 5) supplemented with NMR pH indicators (15 mM sodium citrate, 100 mM NaCl, 0.1 mM EDTA, 4 mM dichloroacetic acid, 0.5 mM dimethylsulfoxide, 6 mM imidazole, 2 mM piperazine, and 4 mM sodium fluoride). HdeA was expressed and purified as described previously (17).

Chaperone Activity Assays—The influence of purified HdeA and HdeB on the aggregation of guanidine hydrochloride (GdnHCl)3-denatured or thermally unfolding malate dehydrogenase (MDH) at different pH values was described as monitored at pH 7 (17, 21, 23). In brief, 150 μM MDH from porcine heart mitochondria (Roche Applied Science) was incubated overnight in 5.4 M GdnHCl, 50 mM potassium phosphate, and 50 mM NaCl (pH 7.5) at room temperature. Guanidine-denatured MDH was then diluted to a final concentration of 2 μM into buffer B (150 mM potassium phosphate, 150 mM NaCl, and 150 mM (NH4)2SO4) at the indicated pH that was pre-equilibrated at 25 °C in the presence and absence of various concentrations of HdeB and HdeA, respectively. All listed concentrations of HdeA and HdeB refer to the monomer concentration. After 20 min of incubation, the pH was rapidly raised to 7 by the addition of 0.16–0.34 volume of 2 M K2HPO4. Thermal aggregation of MDH was monitored at 43 °C. MDH in buffer A was diluted to a final concentration of 0.5 μM into prewarmed buffer C (150 mM potassium phosphate and 150 mM NaCl) at the indicated pH in the presence and absence of 12.5 μM HdeB. After 20 min of incubation, the pH was rapidly raised to 7 by the addition of 0.16–0.34 volume of 2 M unbuffered K2HPO4. Thermal aggregation of rabbit muscle lactate dehydrogenase (LDH; Roche Applied Science) was monitored by light scattering at 41 °C. LDH in 40 mM HEPES and 50 mM NaCl (pH 7.5) was diluted to a final concentration of 1 μM into prewarmed buffer C at the indicated pH in the presence and absence of various concentrations of HdeB. After 5 min of incubation, the pH was raised to 7 by the addition of 0.16–0.34 volume of 2 M K2HPO4. Changes in absorbance due to the light scattering of protein aggregates were monitored at 430 nm using either a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Santa Clara, CA) or a R-4500 fluorescence spectrophotometer (Hita- chi, Tokyo, Japan) equipped with temperature-controlled sample holders. HdeA and HdeB activities were normalized to the light-scattering signal of MDH/LDH in the absence of chaperones at each indicated pH.

MDH Unfolding, Refolding, and Activity Assay—The influence of HdeB on the refolding of acid-denatured MDH was analyzed according to Tapley et al. (21). In brief, 1 μM MDH was incubated in buffer C at the indicated pH for 1 h at 37 °C in the absence or presence of 25 μM HdeA or HdeB, followed by a 10-min temperature equilibration at 20 °C. Subsequently, the samples were neutralized to pH 7 by the addition of 0.5 M HCl.

3 The abbreviations used are: GdnHCl, guanidine hydrochloride; MDH, malate dehydrogenase; LDH, lactate dehydrogenase; bis-ANS, 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid.
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\[ \text{water suppression by gradient-tailored excitation scheme} \]

Results

HdeB Partially Unfolds and Dissociates into Monomers at Low pH—Previous studies suggested that HdeA is activated by pH-induced dissociation and partial unfolding of the monomers (17). The midpoint of HdeA dissociation/unfolding obtained by both bis-ANS fluorescence and FRET measurements was found to be around pH 3.4, consistent with its moderate in vitro chaperone activity at pH 3 and high chaperone activity at pH 2 (11, 17, 23). More recent studies using a condi-
tionally active HdeA mutant revealed that the HdeA dimer is stabilized by electrostatic interactions between aspartic and glutamic acid residues on one monomer and positively charged lysine residues on the other monomer (23, 30). Once the pH shifts below the pKa of the glutamic acid residues, they lose their charge, resulting in the dissociation of the monomers, their partial unfolding, and activation of the chaperone function. In contrast, it has been reported that HdeB has no significant chaperone activity at pH 2 and only modest chaperone activity at pH 3 (10, 22), although fluorescence measurements suggested a HdeA-like midpoint of dissociation/unfolding at pH 3 (10). These results suggested that HdeB displays significantly lower pKa of the glutamic acid residues, they lose their charge, resulting in the dissociation of the monomers, their partial unfolding, and activation of the chaperone function. In contrast, it has been reported that HdeB has no significant chaperone activity at pH 2 and only modest chaperone activity at pH 3 (10, 22), although fluorescence measurements suggested a HdeA-like midpoint of dissociation/unfolding at pH 3 (10). These results suggested that HdeB displays significantly lower α-helical content compared with HdeA. Analysis of the secondary structure content using CDSSTR (31) predicted an ~16% lower α-helical content in HdeB compared with HdeA at pH 7. These results suggested that HdeB is significantly less structured and/or more flexible at neutral pH compared with HdeA, a notion that agreed well with the fact that crystallization of HdeB (but not HdeA) was successful only upon reductive methylation (15), a method known to stabilize protein structures (32). Far-UV CD spectra revealed also no major differences in the secondary structure of HdeB upon exposure to pH 4 (data not shown) or pH 3 (Fig. 1B, dashed line). However, as reported previously (17) and similar to HdeA, partial unfolding of HdeB occurred upon exposure to pH 2 (Fig. 1B, dotted line). This unfolding of HdeB was found to be fully reversible, and HdeB regained its original structure upon shifting the pH from 2 to 7 (data not shown).

To characterize the structural properties of HdeB in more detail, we next conducted analytical ultracentrifugation experiments at different pH values. We found that HdeB sediments predominantly in its dimeric form (s = 1.5 × 10^{-13} S) at pH 7, predominantly in its monomeric form (s = 1.23 × 10^{-13} S) at pH 2, and as a mixture of dimers and monomers at pH 3 (Fig. 1C). These results agreed well with previously conducted fluorescence studies that used the fluorescence quenching of two tryptophan residues in the dimer-dimer interface of HdeB to
monitor its pH-induced dissociation (15). However, to our surprise, we observed substantial changes in the sedimentation behavior of HdeB between pH 7 and 4, where the sedimentation coefficient $s$ of HdeB dimers increased as the pH approached 4 (Fig. 1C), potentially indicative of a higher HdeB volume due to structural rearrangements at pH 4 compared with pH 7. This result was also consistent with surface hydrophobicity measurements using bis-ANS fluorescence and intrinsic tryptophan fluorescence measurements of HdeB (Fig. 1, D and E), which showed substantial structural rearrangements in HdeB between pH 4 and 7.

**HdeB Shows Maximal in Vitro Chaperone Activity at pH 4** — To investigate the influence of pH on the chaperone activity for HdeB, we systematically analyzed the influence of HdeB on the aggregation of several substrate proteins in vitro at different pH values. First, we studied the influence of HdeB on the model substrate porcine mitochondrial MDH at different pH values using either chemically denatured (i.e. GdnHCl) or thermally denatured MDH. We prepared GdnHCl-denatured MDH and diluted it into reaction buffer with or without chaperones at pH 2–5. Independent of the absence or presence of functional chaperones, MDH remains in an unfolded yet soluble state under these low pH conditions (21). Once neutralized, however, MDH rapidly aggregated unless functional chaperones were present during the low pH incubation period (Fig. 2A, solid lines). As observed before, GdnHCl-denatured MDH was fully protected by HdeA at pH 2 and 3 (17, 21, 23), and no significant MDH aggregation was observed upon neutralization under these low pH conditions (Fig. 2A, dashed lines). At higher pH, however, HdeA almost completely failed to protect MDH from aggregation. In contrast, the presence of HdeB did not affect aggregation of MDH upon its neutralization from pH 2, moderately reduced MDH aggregation upon its neutralization from pH 3 or 5, and almost completely suppressed aggregation upon neutralization from pH 4 (Fig. 2A, dotted lines). These results suggest that HdeB chaperone function has a pH optimum around 4, which is in a significantly higher pH range than reported previously (10, 22) and higher than that of HdeA. Fur-

**FIGURE 2. Chaperone activity and refolding ability of HdeA and HdeB at acidic pH.** A, GdnHCl-denatured MDH was diluted to a final concentration of 2 $\mu$M into buffer B at the indicated pH and incubated for 20 min in the absence (solid line) or presence of 20 $\mu$M HdeA (dashed line) or 50 $\mu$M HdeB (dotted line). After raising the pH of the samples to 7 by the addition of 0.16–0.34 volume of 2 M unbuffered K$_2$HPO$_4$ (as indicated by the arrows), MDH aggregation was measured by monitoring light scattering at 350 nm. RLU, relative light units. B, 0.5 $\mu$M MDH was incubated in prewarmed buffer C at the indicated pH in the absence or presence of various concentrations of HdeB. The time course of MDH aggregation upon neutralization was determined as described for A, and the relative chaperone activity was calculated. The activity of HdeA at pH 2 or HdeB at pH 4 was set to 100%. C, 1 $\mu$M LDH was incubated for 5 min at 41°C in prewarmed buffer C at the indicated pH in the absence or presence of various concentrations of HdeB. LDH aggregation upon neutralization from pH 2 (○), pH 3 (■), or pH 4 (□) was measured as described, and the relative chaperone activity was calculated. D, 1 $\mu$M MDH was incubated in buffer C at the indicated pH for 1 h at 37°C in the absence or presence of 25 $\mu$M HdeA or HdeB. The temperature was then shifted to 20°C for 10 min before the samples were neutralized to pH 7 by the addition of 0.5 M Na$_2$HPO$_4$. Aliquots were then taken before and after 2 h of incubation at 20°C and assayed for MDH activity. MDH activity upon neutralization in the absence of chaperones (gray bars) or in the presence of either HdeA (white bars) or HdeB (black bars) is shown. The mean ± S.D. derived from at least three independent measurements is shown.
thermore, HdeB becomes active as a chaperone substantially before monomerization and partial unfolding occur (Fig. 1). To exclude that the folding state of the client protein affects HdeB function, we analyzed the chaperone activity of HdeB at different pH values using thermally denatured MDH as client. For these experiments, native MDH was diluted into 43 °C buffer at the indicated pH with or without HdeA or HdeB present. As before, aggregation of MDH was triggered by neutralizing the reaction buffer at 43 °C. Consistent with our results using chemically denatured MDH, we found that HdeA prevented aggregation of thermally unfolding MDH upon neutralization from pH 2 or 3 (Fig. 2B, white bars), whereas HdeB reduced MDH aggregation upon its neutralization from pH 4 or 5 (black bars). Very similar results were also obtained when we used thermally unfolding LDH, where the presence of a 20-fold excess of HdeB had no effect on the aggregation of LDH at pH 2, whereas a 2-fold molar excess of HdeB significantly reduced aggregation upon LDH dilution at pH 4 (Fig. 2C). We concluded from these results that independent of the client proteins or the method of client unfolding, HdeB appears to have its optimal chaperone activity around pH 4 and that this optimum is significantly above the pH range at which monomerization and unfolding occur (pH < 4).

**Fig. 2D.** In the absence of HdeA or HdeB, acid-denatured MDH had a very low propensity for spontaneous refolding, resulting in <1% MDH activity independent of the original pH conditions (Fig. 2D, gray bars). Consistent with previous reports (21), we found that in the presence of HdeA, substantial reactivation of MDH was achieved upon neutralization from pH 2 or 3, but not upon neutralization from pH 4 and 5 (Fig. 2D, white bars). In the presence of HdeB, however, significant reactivation of MDH was achieved only upon neutralization from pH 4 (Fig. 2D, black bars). We concluded from these results that much like HdeA, HdeB is able to protect proteins against pH-induced aggregation and facilitates their reactivation upon neutralization. The major difference between these two proteins lies within their optimal pH at which they fulfill this important task.

**HdeB Dimers Form Complexes with Client Proteins at pH 4—** Our previous results suggested that in contrast to HdeA, which is chaperone-active when monomeric and partially unfolded, HdeB works in its dimeric conformation. To directly monitor the complex formation between HdeB and client proteins, we used analytical ultracentrifugation analysis. We incubated HdeB and its client protein LDH at either pH 7 or 4 for 15 min at 41 °C, cooled the reaction down, and performed analytical ultracentrifugation experiments at 22 °C. Upon incubation of the two proteins at pH 7, we did not observe any complex formation (Fig. 3A). As in the absence of additional proteins, HdeB at 22 °C sedimented predominantly in its dimeric state (s = 1.45 × 10⁻¹³ S) (Fig. 3, A and C), indicating that elevated temperatures do not affect its oligomerization state at pH 7 or 4. LDH was present exclusively as a tetramer, the biologically active state, confirming the observation that LDH was not sub-

![Figure 3. Complex formation of HdeB dimers with unfolded LDH at pH 4 by analytical ultracentrifugation.](Image)

LDH (3 μM) was incubated in the presence of a 10-fold molar excess of HdeB in buffer C for 15 min at 41 °C and pH 7 (A) or pH 4 (D). For comparison, LDH alone (B) or HdeB alone (C) was incubated for 15 min at 41 °C and pH 4. The results from analytical ultracentrifugation sedimentation velocity analysis are shown. Sedimentation coefficient distribution (c(s)) was analyzed using SEDFIT. LDH₄, LDH tetramer; LDH₀, LDH monomer; HdeB₀, HdeB dimer; HdeB-LDH₄, HdeB-LDH complex.
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**TABLE 2**

Summary of analytical ultracentrifugation data

| Sample | Oligomeric state | Mass (kDa) |
|--------|------------------|------------|
| Fig. 3A | LDH + HdeB (pH 7) | HdeB dimer | 16.4 |
| Fig. 3B | LDH (pH 4) | Monomer | 44.1 |
| Fig. 3C | HdeB (pH 4) | Dimer | 18.2 |
| Fig. 3D | LDH + HdeB (pH 4) | HdeB dimer | 18.3 |
| | | LDH-HdeB complex | 134 |

We previously showed that HdeA and HdeB are different in their interaction with bacteria. We found HdeB to be at least as effective as HdeA in protecting bacteria against acid stress (19). In this study, we further explored the reason behind the differential protective capacity of these two proteins in an attempt to understand their function in the periplasm of E. coli.

Our experiments were performed in vitro, and it is therefore important to take into account the potential differences due to the fact that in vivo conditions are more complex. However, our results showed HdeB to be a more effective chaperone than HdeA under conditions of acid stress.

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HdeB appears to be active at pH 2–3, whereas HdeA is active at pH 4. This is consistent with our previous findings and suggests that HdeB is more effective in protecting bacteria against acid stress.

**New Findings**

We found that HdeB is more effective in protecting bacteria against acid stress than HdeA. This is in contrast to our previous findings, where we found HdeA to be more effective.

**Future Directions**

Further studies are needed to understand the mechanisms by which HdeB and HdeA interact with bacteria and how they protect them against acid stress.

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peared from the spectra (Fig. 5, lower panel). This transition likely corresponds to the monomerization of HdeB, causing at least partial unfolding, as suggested by the decrease in the range of observed chemical shifts. Additional experiments, including the assignment of the HdeB spin system, are now necessary to further define the changes in HdeB that contribute to its activation. However, we can conclude from these studies that the conformational properties of HdeB are highly pH-sensitive at a pH range that coincides with its activation as a chaperone. At neutral and very low pH, however, slower conformational transitions appear, potentially defining the limit of HdeB chaperone activity.

DISCUSSION

Many proteins are susceptible to acid-mediated denaturation. The presence of pores in the outer membrane of Gram-negative bacteria thus makes the periplasmic proteome a vulnerable target of acid stress in bacteria (10, 16, 34). To chaperone protein folding under these low pH conditions, enteric bacteria such as *E. coli* encode several stress-specific chaperones, including HdeA, whose acid-induced activation protects proteins against the otherwise lethal protein aggregation. Triggered by a downshift to pH <3, HdeA monomerizes and partially unfolds, allowing it to bind and protect other acid-denatured proteins as long as the pH remains low (16, 17, 21, 23). Upon neutralization, HdeA slowly releases its client proteins. This mechanism keeps the concentration of aggregation-sensitive intermediates low and facilitates protein refolding (21). It has been previously shown that HdeA is only modestly active at pH 3 and inactive at pH 4, raising the question about how *E. coli* cells withstand moderately acidic stress conditions. In this study, we investigated HdeB, a structural homolog of...
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With these findings, we now present a working model illustrating how the acid-activated chaperones HdeA and HdeB assist during acid stress and recovery in E. coli (Fig. 6). At neutral pH, both HdeA and HdeB are present as well folded dimers. Upon a shift to pH 2, as usually occurs upon entering the mammalian stomach, HdeA and HdeB rapidly monomerize and partially unfold. Whereas HdeB is reversibly inactivated under these acid-stress conditions, HdeA undergoes structural changes that allow it to tightly interact with unfolding client proteins and inhibit their acid-induced aggregation. Upon transition back to pH 7, as occurs between the stomach and small intestine, proteins can now directly refold upon release from HdeA. A slow transition to neutral pH might cause the inactivation of HdeA and the subsequent release of the substrates at pH >3. At pH 4–5, HdeB may then bind the released substrates to prevent them from aggregation. HdeB might have a more physiologically important role during extended fasting periods, when the pH of the mammalian stomach is increased to pH 4 (3). These conditions are still acidic enough to be denaturing but no longer activate HdeA. Then, HdeB can take over the function of HdeA and promote protein protection. The presence of both HdeA and HdeB therefore appears to enable E. coli cells to rapidly respond to a variety of acid stress conditions at a

HdeA, and the second E. coli protein suggested to protect periplasmic proteins against acid stress (10, 22). We presented evidence that HdeB is a molecular chaperone that functions at a pH range that is still potentially bactericidal but significantly higher than the pH range in which HdeA is optimally active. In vitro chaperone studies using different client proteins established that HdeB exhibits optimal chaperone activity around pH 4–5. Our studies show that similar to HdeA, HdeB is a dimer at neutral pH and dissociates into monomers at pH 2–3, which is accomplished by at least partial unfolding of the monomers. However, unlike for HdeA, which is partially unfolded at its optimal pH of <3, activation of HdeB clearly precedes acid-induced monomerization. These results suggest that the hydrophobic surface of the dimerization interface, which has been the proposed client-binding site in HdeA (17), is unlikely to serve for substrate binding in HdeB. The unifying feature between the two proteins, which share high structural but very limited sequence homology, may be a pH-mediated increase in flexibility that triggers their chaperone activity. In fact, our NMR, fluorescence, and analytical ultracentrifugation studies demonstrated that HdeB is a very dynamic protein and suggested substantial structural rearrangements upon reaching the activating pH of 4. These results suggest that the structural changes that we observed between pH 4 and 7 are sufficient for activation of HdeB chaperone function. It now remains to be tested whether the activation mechanisms of HdeA and HdeB are fundamentally different or whether activation of both proteins involves similar, potentially more local rearrangements, which, in the case of HdeA, coincide with and are masked by the more global unfolding events. Future studies may involve constant pH molecular dynamics calculations based on the crystal structure of HdeB. This approach was successfully used to identify amino acid residues crucial for the acid-induced activation of HdeA (23).

With these findings, we now present a working model illustrating how the acid-activated chaperones HdeA and HdeB assist during acid stress and recovery in E. coli (Fig. 6). At neutral pH, both HdeA and HdeB are present as well folded dimers. Upon a shift to pH 2, as usually occurs upon entering the mammalian stomach, HdeA and HdeB rapidly monomerize and partially unfold. Whereas HdeB is reversibly inactivated under these acid-stress conditions, HdeA undergoes structural changes that allow it to tightly interact with unfolding client proteins and inhibit their acid-induced aggregation. Upon transition back to pH 7, as occurs between the stomach and small intestine, proteins can now directly refold upon release from HdeA. A slow transition to neutral pH might cause the inactivation of HdeA and the subsequent release of the substrates at pH >3. At pH 4–5, HdeB may then bind the released substrates to prevent them from aggregation. HdeB might have a more physiologically important role during extended fasting periods, when the pH of the mammalian stomach is increased to pH 4 (3). These conditions are still acidic enough to be denaturing but no longer activate HdeA. Then, HdeB can take over the function of HdeA and promote protein protection. The presence of both HdeA and HdeB therefore appears to enable E. coli cells to rapidly respond to a variety of acid stress conditions at a

FIGURE 5. pH-induced conformational changes in HdeB followed by NMR. Shown are 1H-15N heteronuclear single-quantum coherence signals of HdeB at various pH values: pH 6.8 (purple), pH 5.6 (coral), pH 5.1 (orange), pH 4.5 (yellow), pH 3.8 (green), pH 2.8 (cyan), and pH 2.2 (blue).
broader pH range, minimizing the irreversible aggregation of acid-unfolded proteins.

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FIGURE 6. Model for the mechanism of the acid-activated chaperones HdeA and HdeB. At pH 7, both HdeA (red) and HdeB (blue) are inactive dimers. Upon a shift to pH 2, as occurs in the mammalian stomach, HdeA and HdeB rapidly monomerize and partially unfold (arrow a). Whereas HdeB is inactive in this form, partially unfolded HdeA monomers tightly bind to unfolding client proteins (green) and inhibit their acid-induced aggregation. Fast neutralization (arrow b) triggers the release of unfolded substrate proteins, which subsequently refold. Upon slow neutralization (pH 4), HdeA and HdeB both refold. Whereas HdeA is inactive in this state, HdeB is now active and could potentially bind the unfolded client proteins until neutral pH conditions are restored. During fasting periods (arrow c), the pH of the mammalian stomach is increased to pH 4. Under these conditions, only HdeB is active and protects client proteins from pH 4-mediated protein unfolding and aggregation.
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