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Continuous and Pulsed Hydrogen-Deuterium Exchange and Mass Spectrometry Characterize CsgE Oligomerization

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Abstract

We report the use of hydrogen-deuterium amide exchange coupled to mass spectrometry (HDX-MS) to study the interfaces of and conformational changes accompanying CsgE oligomerization. This protein plays an important role in enteric bacteria biofilm formation. Biofilms provide protection for enteric bacteria from environmental extremes and raise concerns about controlling bacteria and infectious disease. Their proteinaceous components, called Curli, are extracellular functional amyloids that initiate surface contact and biofilm formation. The highly regulated curli biogenesis involves a major subunit, CsgA, a minor subunit CsgB, and a series of other accessory proteins. CsgE, possibly functioning as oligomer, is a chaperonin-like protein that delivers CsgA to an outer-membrane bound oligomeric CsgG complex. No higher-order structure, or interfaces and dynamics of its oligomerization, however, are known. In this work, we first determined regions involved in CsgE self-association by continuous HDX, and, on the basis of that, prepared a double mutant W48A/F79A, derived from interface alanine scan, and verified that it exists as monomer. Using pulsed HDX and MS we observed an interesting structural rearrangement that occurs during the oligomerization of CsgE.

Keywords

Curli; oligomerization interface; hydrogen/deuterium exchange-MS (HDX); EX1-like HDX; pulsed HDX; continuous HDX; CsgE

Introduction

Curli are the first identified functional amyloid fibers produced by *Escherichia coli*, and other enteric bacteria. Curli fibers adhere to a host's surface and are critical for biofilm formation.^{1–3} Bacteria within biofilms are protected from environmental extremes (e.g., disinfectants, antibiotics), allowing many infections to re-occur.⁴ Thus, curli proteins are a target for treatment of many persistent and chronic bacterial infections.⁵ Understanding this highly regulated, amyloid production process is not only important in infectious disease

Supporting Information

The supporting information is available free of charge via the Internet at <http://pubs.acs.org>.

management but also in providing a perspective on molecular mechanisms underlying Alzheimer's, Parkinson's, Huntington's, and other human neurodegenerative diseases that are caused by misfolded amyloids.⁶

The highly regulated biogenesis of curli fibers is encoded by two divergently transcribed *csg* (curli-specific gene) operons, *csgBAC* and *csgDEFG*.^{1, 7} Operon *csgBAC* is regulated by the transcriptional factor CsgD.⁷ CsgA, the major curli subunit, is secreted as an intrinsically disordered protein to the outer membrane surface and rapidly forms amyloid aggregates upon interacting with CsgB, the nucleator and a minor curli subunit.^{8, 9} CsgC stabilizes periplasmic CsgA and efficiently inhibits CsgA aggregation.¹⁰ Membrane-bound CsgG associates into a pore-like nonamer that is required for curli secretion and localization.^{11–13} CsgF, a surface-exposed chaperonin-like protein, assists in directing and tethering curli amyloid to the cell surface by interacting with both CsgG and CsgB.¹⁴ CsgE is another chaperonin-like protein that prevents CsgA amyloid formation within the periplasm and delivers CsgA to the pore CsgG for secretion *in vivo*.¹⁵ CsgE inhibits amyloid formation of CsgA *in vitro*.^{15, 16} Little else is known about the biophysical properties of CsgE except that it may form a nonameric adaptor that binds to the membrane-extracted nonameric CsgG complex.¹² It is possible that CsgE functions in this oligomeric state rather than as monomer. Therefore, the structural characterization of CsgE and its oligomerization-induced conformational changes are critical to elucidate the role of CsgE in curli biosynthesis.

Given the tendency of CsgE to aggregate, high resolution methods including X-ray crystallography and NMR are difficult, if not impossible, to apply. Here, we utilized continuous and pulsed methods of hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) to study regions of self-association, and we followed the conformational changes during the protein's extensive oligomerization. We chose HDX because it is useful in probing protein conformation and dynamics.^{17–21} Protein backbone amide hydrogens exchange with deuterium based on solvent accessibility and hydrogen bonding. Thus, protein structural information is coupled to a mass shift that is easily detected by mass spectrometry. In conventional and widely employed continuous HDX, the protein is diluted into D₂O buffer for various times prior to measurement of deuterium uptake. The increased uptake, as a function of exchange time, reflects dynamics of backbone amide hydrogen bonds and solvent accessibility, and, therefore, provides insights into protein conformation.^{17, 18} On the other hand, pulsed HDX examines a short HDX time window after perturbing the protein for different periods of time. In this case changes in deuterium uptake represent distinct populations of molecules, each of which has different exchange profiles. Therefore, protein structural change is probed in a complementary way to continuous HDX.^{22, 23}

Materials and Methods

Protein Expression and Purification

Wild type CsgE was expressed with a C-terminal 6xHis-tag and without the signal peptide 1-21 by using pNH27 in strain NEB 3016 with 100 µg/mL ampicillin, as reported previously.¹⁵ The substitution, tryptophan 48-to-alanine (W48A) was first introduced into the wild type CsgE by using the QuikChange, site-directed mutagenesis kit (Stratagene, La

Jolla, CA). A substitution of phenylalanine 79 to alanine (F79A) was further introduced into W48A to produce the double mutant W48A/F79A. All constructs were sequenced with BigDye Terminator v3.1 cycle sequencing kit and the 3730 DNA Sequencers (Applied Biosystems, Foster City, CA) to ensure their integrity and accuracy.

Both WT and mutant CsgE were expressed as previously described.^{15, 24} The proteins were purified by using two steps of FPLC. Any precipitate that formed during purification was removed by centrifugation, and the supernatant was filtered through a 0.22 μ m membrane before loading on to the column. The lysis of cell pellets was done in 20 mM imidazole, 0.5 M NaCl, 50 mM potassium phosphate, pH 8.0. After centrifugation and filtration, the supernatant was applied to affinity FPLC by using a nickel affinity column, HisTrap FF Crude (GE Healthcare, Buckinghamshire, UK). The protein was washed with lysis buffer and eluted with a solution of 500 mM imidazole, 500 mM NaCl, 50 mM potassium phosphate (pH 7.4). The protein in the eluate was further purified by size-exclusion FPLC by using a HiPrep 26/60 Sephacryl S-100 HR column (GE Healthcare) and 50 mM potassium phosphate (pH 7.4), 150 mM NaCl, 500 mM arginine. Purified CsgE was stored at 4 °C before concentrating and exchanging the buffer for further analysis. Trace amount of arginine possibly exists in solution after buffer exchange. The protein concentration was determined by absorbance at 280 nm on a Varian Cary 50 Bio UV/Vis spectrophotometer (Palo Alto, CA) by using 1 mL volume and molar absorptivities of 19,460 and 13,980 M⁻¹ cm⁻¹ for wild type and mutant, respectively.

Materials

All chemicals other than the proteins were purchased from Sigma-Aldrich (St. Louis, MO). D₂O was from purchased from Cambridge Isotope Laboratories Inc. (Andover, MA).

HDX Protocol

Continuous HDX was initiated by diluting CsgE stock solution in phosphate buffer saline (PBS), pH 7.4, 1:3 into D₂O buffer (PBS, pD 7.4) at 4 °C. The 1:3 dilution factor was chosen so that a relatively high protein concentration could still be maintained after D₂O dilution without concentrating the stock solution and causing aggregation during preparation or sample storage. At various incubation times (10 s, 30 s, 1 min, 5 min, 15 min, 1 h, 4 h), HDX was quenched by adding 3 M urea/1% (v/v) trifluoroacetic acid (TFA) at 2:3 volume ratio. For establishing a back exchange correction, 0.75 μ M WT CsgE with 5 mM Arg or the CsgE W48A/F79A mutant was incubated in D₂O buffer at 4 °C for 72 h.

For pulsed HDX, 10 μ M CsgE (PBS, pH 7.4) was incubated at 25 °C with continuous stirring to promote CsgE oligomerization. Pulsed HDX was performed by mixing the incubated sample 1:5 into D₂O buffer (PBS, pD 7.4) at 4 °C for 30 s, and then quenched the same way as for continuous HDX.

LC-ESI/MS Analysis

The quenched solution was immediately injected into a custom-built, on-line sample-handling device that was immersed in a water-ice (0 °C) bath to minimize back exchange, as previously described.²⁵ Briefly, the protein solution was digested by passing it through an

immobilized pepsin column (2 mm × 2 cm) at 200 µL/min in 0.1% TFA via an Agilent 1100 HPLC (Santa Clara, CA), and the resulting peptic peptides were trapped and desalted on a C8 trap column (2.1 mm × 1.5 cm, Agilent, Santa Clara, CA) for 3 min. The peptic peptides were then eluted from the trap column and separated on a C18 analytical column (1.9 µm Hypersil Gold, Thermo Fisher, Waltham, MA) with a linear gradient (4–40% CH₃CN, 0.1% (v/v) formic acid) at 50 µL/min delivered by a Waters nanoACQUITY UPLC (Milford, MA) for 5 min. All measurements were done in triplicate.

HDX Data Analysis

Preliminary to HDX, peptides from pepsin digestion were identified in a data-dependent mode on a Thermo LTQ XL Orbitrap (San Jose, CA). The six most abundant ions were submitted to collision-induced dissociation (CID) fragmentation. Product-ion spectra were then submitted to MassMatrix (version 2.4.2) for identification,²⁶ and manually inspected. Only verifiable peptides were used for the HDX analysis. HDX data were collected on a MaXis quadrupole time of flight (Bruker, Bremen, Germany) mass spectrometer in the positive-ion electrospray ionization mode. The settings were: capillary voltage, 3.8 kV; nebulizer gas, 0.8 bar; drying gas flow rate and temperature, 6 L/min and 180 °C, respectively. Data analysis of continuous HDX was carried out with HDExaminer (version 1.1.0, Sierra Analytics, Inc., Modesto, CA). EX1-like or EXX-like kinetics were fit by a customized program implemented in MathCAD (v14, Parametric Technology Corp., MA).

Thermal Denaturation by Circular Dichroism

The far-UV circular dichroism (CD) spectra were measured on a Jasco-715 spectropolarimeter (Tokyo, Japan) equipped with thermoelectric temperature control. The CD melting curves were recorded as a function of temperature at 222 nm to report structural unfolding. The temperature change was from 20 to 80 °C at a rate of 1 °C/min. The protein concentration was 10 µM in 10 mM potassium phosphate buffer, pH 7.4.

Analytical Ultracentrifugation

A Beckman Optima model XL-A analytical ultracentrifuge (Brea, CA) was used for the sedimentation velocity experiments. Samples were loaded into a conventional double-sector filled epon centerpiece (path length 1.2 cm). Data were collected in continuous mode at 290 nm. Experimental conditions: 50 mM potassium phosphate, pH 7.4, 150 mM NaCl at 25 °C at 20 µM CsgE. Data were analyzed using Sedfit.²⁷

Results and Discussion

Self-assembly of proteins into different oligomeric states is a complex process that is likely to be protein-dependent and remains difficult to understand.^{28, 29} Therefore, it is necessary to start this section with the design of the oligomerization study reported here. First, we studied CsgE by continuous HDX and identified regions involved in self-association. Considering that CsgE is prone to aggregate at 25 °C, we conducted our HDX experiments at 4 °C to maintain the protein oligomeric state the same within the experimental window. The protein is likely restricted to mainly monomer and a fraction of oligomers at this low temperature. It is also known that quaternary structural rearrangements derived from

oligomerization significantly affect protein function and play a vital role in biochemical processes.³⁰ Curli are expressed in a stationary phase at temperatures below 30 °C.³¹ Thus, we are also interested in investigating the oligomerization-induced conformational changes under nearly biologically relevant temperature (i.e., 25 °C), to improve our understanding of the role of CsgE in curli biogenesis. Continuous HDX is not applicable here, however, as both oligomerization and HDX are time-dependent processes, and the results are a convolution of both. Thus, we applied pulsed HDX, a platform implemented by us previously for A β ₄₂²³, to study CsgE conformational changes accompanying extensive oligomerization.

Structural and interface information from continuous HDX

We conducted comprehensive differential HDX analysis of CsgE at different concentrations and at 4 °C to determine the oligomerization interfaces of the protein. We do not know, however, the concentration CsgE exists as monomer. Arginine can suppress protein aggregation.³² We found that adding arginine at mM concentrations results in a monomer-predominant CsgE solution possibly by interrupting various hydrophobic interactions. Therefore, we performed HDX on 0.75 μ M CsgE WT in the presence of 5 mM arginine (WT_{Arg}) to help identify the self-association regions. We corrected for back exchange to assure a more accurate interpretation of the results. In our study, 99% of the CsgE could be covered with multiple overlapping peptides formed by on-line pepsin digestion. We selected 12 peptides to represent the HDX kinetics over the full protein (Figure 1). The complete set of kinetic curves can be found in *Supporting Information* (Figure S1).

Considering there is currently no report of a CsgE structure, we first studied deuterium uptake of 0.75 μ M CsgE WT (green, Figure 1) to obtain broad structural information. HDX provides insights into structure because regions with secondary structure undergo slower HDX than do loops or unstructured regions.^{17, 18} Deuterium uptake of peptides 4-9, 89-97 and 98-114 reached maximal exchange rapidly, suggesting that those regions located at the N- and C-termini are highly dynamic and unstructured. In contrast, less than 5% HDX occurred for peptides 59-62 and 80-88 over the entire time course. This extraordinarily slow exchange behavior indicates that either these two regions contain rigid secondary structural elements or the regions are located at a central core of the structure. HDX extents at regions represented by peptides 11-22, 23-38, 39-51, 63-66, and 67-79 were between these two extremes, indicating some locally defined secondary structures. Deuterium uptake of peptide 52-58 remained constant at about 40%, and this is likely a result of composite behavior caused by partially unstructured and partially rigid structures in this region of the protein, given that back exchange has already been corrected.

We next compared the time-dependent HDX of each peptide under the following conditions: 0.75 μ M WT_{Arg} (red), 0.75 μ M WT (green), 5 μ M (cyan), 33 μ M (purple), and 66 μ M (black) (Figures 1 and S1). There are regions that are unaffected by oligomerization occurring under these conditions. We view those regions, 4-9, 52-58, 59-62 and 98-114, as a negative control; HDX in those regions was not affected by protein concentration or the presence of arginine. To identify the critical regions in self-association, we made two comparisons: one is the HDX difference between 0.75 μ M WT_{Arg} (red) and 0.75 μ M WT

(green), and the other is the difference between WT at 0.75 μM (green) and at 5 μM (cyan), 33 μM (purple) and 66 μM (black) (Figures 1 and S1). In the first comparison, differential information is obtained between monomer and oligomers. Major differences for peptides 26-33, 39-51 and 67-79 occurred at short HDX times but diminished at longer times. We interpret this to be due to equilibrium dissociation of oligomers and faster HDX in their monomeric forms. A small but statistically significant difference (student's t-test at 95% confidence interval) for peptide 23-38 was observed over the entire time course, further supporting that 26-33 is an important interface. Apparent differences in HDX also occurred for peptides 11-22 and 80-88 at longer time points (after 1 h), but not at shorter time points. This phenomenon indicates that long-term dynamics and protein breathing, instead of solvent exposure of these regions, are mostly affected.

In the second comparison no arginine is involved. Here, the HDX profiles give an integrated picture of equilibrium between monomers and different oligomers in solution. Presumably, higher protein concentrations increase the average size of oligomers present in solution. More extensive HDX of peptides 26-33, 39-51 and 67-79 was again observed at 0.75 μM than at higher concentration for most time points, while the HDX difference for peptide 63-66 became significant only after 1 h incubation. The results from the two comparisons suggest that regions 26-33, 39-51 and 67-79 are important oligomerization interfaces. There is a possibility, however, that other critical regions in self-association are not clearly revealed by our data. It is difficult to tell whether small differences of deuterium uptake are directly from oligomerization interfaces or the result of an allosteric effect where conformational changes occur remote from the oligomer interfaces. Nevertheless, continuous HDX provides useful information on CsgE structure and strongly suggests the self-association regions.

A Monomeric Mutant

To confirm further that regions identified above contain critical residues involved in self-association, we substituted alanine for certain amino acids located at the putative interfaces. Among all the substituted proteins, we found that the W48A/F79A formed by a double mutation is significantly more stable than the WT *in vitro*, as measured by thermal denaturation (Figure 2). Using analytical ultracentrifuge experiments of this mutant, we found a single species with a calculated molecular weight of 12 - 13 kD essentially the same as the theoretical molecular weight of 12.3 kDa (Figure S2). We monitored circular dichroism signals at 222 nm, indicative of α -helices, as a function of temperature for both WT (blue) and W48A/F79A (red). The denaturation curve for W48A/F79A was shifted to higher temperature relative to that of WT. The T_M values, midpoint of the unfolding transition, of WT and W48A/F79A are 36.7 ± 0.2 and 45.6 ± 0.1 $^{\circ}\text{C}$, respectively, determined by fitting the signal change to a two-states (folded and unfolded) unfolding transition.³³ The increased T_M value indicates that W48A/F79A is more stable than the WT.

Protein stability should correlate inversely with oligomerization propensity.^{34, 35} This offers a means to interpret the continuous HDX on W48A/F79A at 0.75 μM in comparison with the HDX of 0.75 μM CsgE WT and 0.75 μM WT_{Arg} and to test whether W48A/F79A is consistent with the protein being monomeric. Overall, the HDX extents for peptides that are

not involved in self-association are the same for W48A/F79A, WT, and WT_{Arg} (Figure S3), strongly suggesting that substitution of W48A/F79A does not affect overall protein structure and dynamics. The deuterium uptake plots for critical self-association regions (26-33, 39-51 and 67-79) (Figure 3), however, show that for all three regions, HDX of W48A/F79A mutant (blue) is greater when compared to that of WT (green), indicating more exposure or weaker H-bonding at self-association interfaces. This is consistent with the results of our HDX experiments discussed above. Interestingly, significantly higher HDX of W48A/F79A (blue) than that of WT_{Arg} (red) occurs for peptides 39-51 and 67-79, but not for peptide 26-33. Noticeably, two mutation sites are located within peptides 39-51 and 67-79. Although one explanation is that there is some oligomer present for WT_{Arg} but W48A/F79A is a pure monomer, we do not think that is the case because HDX of W48A/F79A for peptide 26-33 is the same as that of WT_{Arg}. The W48A/F79A substitutions reduce local conformational rigidity in regions 39-51 and 67-79 without apparently affecting other regions, resulting in higher deuterium uptake in these two regions. On the other hand, peptide 26-33 shows the same HDX extent as WT_{Arg} because W48A/F79A exists as monomer owing to the interruption of critical self-association residues. This comparison between W48A/F79A, WT and WT_{Arg} further confirms the importance of residues W48 and F79 in CsgE self-association.

Structural Rearrangement Revealed by Pulsed HDX and MS

The recent discovery of a CsgE nonamer¹² leads to a proposal that the CsgE monomer is relatively biologically inactive and must oligomerize to achieve some of its function *in vivo*. Little progress has been made, however, towards understanding CsgE oligomerization. Zhang et al.²³ has demonstrated that pulsed HDX is a useful tool for studying soluble A β as an aggregation system. Here, we applied this method to follow CsgE conformational changes accompanying oligomerization. In this study, CsgE was allowed to oligomerize extensively at 25 °C with continuous stirring. At given incubation times, we used a 30 s pulse of HDX to take a “snapshot” of the *soluble* protein oligomeric states at 4 °C.

First, we compared the HDX of each peptide at given incubation times to that at $t = 0$ (i.e., before starting incubation with continuous stirring). A heat map of the differential uptake between given incubation times and $t = 0$ of representative peptides is shown in Figure 4. In contrast to the aggregation of A β ₄₂, for which deuterium uptake of all peptides decreased (protection increased) in a modified sigmoidal behavior²³, CsgE exhibits some interesting local behaviors. HDX of peptides 4-10, 52-58, 63-66, 67-79, 89-97, and 98-114 showed a significant decrease in HDX (shown in blue) attributable to the accumulation of higher order structures, as expected for an oligomerizing system. Most of the peptides that showed increased protection with oligomerization, with the exception of the N terminus, comprise the C-terminal half of the protein.

Surprisingly, deuterium uptake of peptides of most of the N-terminal half (amino acids 11-51) and 80-88 underwent increased HDX (shown in red), suggesting more open local structures are formed upon oligomerization. These results suggest the various regions play different roles in CsgE oligomerization. Deuterium uptake plots as a function of incubation times of selected peptides, provided in Figure S4, indicate that most significant

HDX changes among all peptides happen within 10 h. This interesting “alternating increase or decrease” of HDX behavior suggest that there is a significant structural rearrangement of the monomer as it oligomerizes. Because peptide-level information obtained from HDX is the average of all species in solution, it is unclear whether the HDX is sampling principally the monomer or soluble oligomers. Nevertheless, both monomer and oligomers must exist along an equilibrium oligomerization pathway, and any monomer in the mixture also reports on the structural rearrangement because monomer $\rightleftharpoons$ oligomer. A possibility is that monomer undergoes conformational rearrangement as it equilibrates to give dimer. The dimer and other small oligomers then form large oligomers in which the structure of the monomer is conformationally isomerized. More evidence is needed to deconvolve HDX properties of the monomer, isomerized monomer, and oligomers, possibly from coupling HDX with chromatography.

Nevertheless, we can look more closely at the oligomerization by examining the deuterium distributions of the various peptic peptides. As expected, most peptides display a single binomial distribution representing deuterium patterns for the peptide molecular ions. Surprising exceptions are peptides encompassing residues 11-22, 23-38 and 89-97, which exhibit broad isotopic envelopes comprised of two and even three deuterium distributions (Figure 5). We fit the mass spectral distributions by using a customized program with two binomial distributions for peptides 11-22, 89-97 and three distributions for peptide 23-38. At least three modes pertain to peptide 23-38 because we could not obtain a satisfactory fit with two modes.

For peptide 11-22, the higher mass distribution (less protected) increases with incubation time whereas the low mass (presumably monomer) decreases. For peptide 89-97, the lower mass (more-protected) distribution appeared in addition to the high-mass (i.e. less-protected) distribution as revealed by comparing the distribution at $t = 0$. The less-protected species is presumably the monomer. For peptide 23-38, two less-protected species appear with incubation time and coexist with the monomeric species. The deuterium uptakes represented by the mass centroids of the various distributions are nearly invariant whereas the relative intensities of the distributions change with incubation time.

Although some of these multiple distributions resemble that of an EX1 HDX mechanism wherein the HDX rate is much faster than protein-closing rate^{36, 37}, we interpret the multiple distributions to indicate the oligomerization-dependent isomerization invoked above. This is not a manifestation of changes in HDX because it is time-independent (the HDX time window is a constant-width pulse). For peptide 11-22, the relative intensities of signals for less-protected structures increased, resulting in a higher centroid mass shift than that at $t = 0$, sharing similar features of the EXX HDX mechanism wherein EX1 and EX2 HDX occur concurrently.³⁸ Peptide 23-38 showed a more complicated isotopic envelope indicating at least two species besides the normal monomer and suggesting that CsgE may exist in two oligomeric forms. The distribution of peptide 89-97, however, is most clearly not that of an EX1. Rather the low mass distribution (more protected) increases with incubation time. The multiple species distribution is also representative of a mix of oligomers of different structures. Given that each peptide is a reporter of the protein states and cannot be

contradictory, we suggest that there are at least three major conformations present in solution during the oligomerization occurring with stirring at room temperature.

The di-substituted protein, W48F/F79A, interestingly and perhaps coincidentally, is modified at the junction of two regions (red and blue, Figure 4). Although peptides in both regions 23-51 and 67-79 are involved in self-association (enclosed in green box), as revealed by continuous HDX, region 23-51 opens and becomes more flexible (less protected) whereas 67-79 closes and shows more protection by pulsed HDX.

Conclusion

Continuous HDX at different concentrations indicates that regions 26-33, 39-51 and 67-79 of CsgE are involved in self-association at 4 °C. This outcome directed effective site-directed mutagenesis and preparation of a monomeric doubly substituted W48A/F79A. Re-examination of the substituted protein demonstrates that HDX not only compares two states (monomer vs. oligomer) but also provides useful guidance for monomeric-mutant design.

Pulsed HDX gives insight on the oligomerization and suggests a significant structural rearrangement occurs accompanying oligomerization, as demonstrated by the presence of a conformationally alternating open and closed structure. Regions involved in self-association at 4 °C, as indicated by continuous HDX, exhibit different conformational changes during more extensive oligomerization at 25 °C; peptides 26-33 and 39-51 become more exposed whereas peptide 67-79 stays more protected. Coupling molecular-weight-based separation with pulsed HDX should elucidate more fully the complex oligomerization pathway. Nevertheless, these results from continuous and pulsed HDX, taken together, provide early insight about CsgE's structure and its conformational changes during oligomerization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Continuous HDX kinetic plots of selected peptic peptides at concentration (after dilution) of 0.75 μM CsgE with 5 mM Arginine (red), 0.75 μM CsgE (green), 5 μM CsgE (cyan), 33 μM CsgE (purple), 67 μM CsgE (black) at 4 $^{\circ}\text{C}$.

Figure 2.

Comparison of thermal denaturation of WT CsgE (blue) and the doubly substituted W48A/F79A (red) monitored by CD at 222 nm. The signal change was fit to a two-state (folded and unfolded) unfolding transition of each form. The T_M values of WT and W48A/F79A are 36.7 ± 0.2 and 45.6 ± 0.1 °C, respectively.

Figure 3. Continuous HDX kinetic plots of peptides at interfaces of 0.75 μ M CsgE double substitution W48A/F79A (blue), 0.75 μ M CsgE with 5 mM Arginine (red) and 0.75 μ M CsgE WT (green). The two substitution sites, W48A/F79A, are located in peptides 39-51 and 67-79, showing higher deuterium uptake than that of CsgE with 5 mM arginine.

Figure 4. Differential Heat Map of pulsed HDX peptic peptides of 8 μM CsgE at 25 °C with continuous stirring. Incubation times are labeled on the left. Each number is the deuterium uptake difference between a certain time point and t = 0 of the same peptide. The color code is given above the sequence. Regions identified in self-association are boxed in green along the protein sequence.

Figure 5.

Mass spectra of peptides showing broad HDX isotopic patterns at different incubation times. Peptides 11-22 and 39-51 are fit with two distributions (red and blue dotted lines) while peptide 23-28 is fit with three distributions (red, blue and green dotted lines). The total fit is represented by black dotted line. A vertical dashed line colored the same of each distribution is included to help directing eyes.