Introduction

Purpose
To determine the interface of the SecA dimer, an important protein transporter in the bacterial cytosol, and to assess whether dimerization causes allosteric effects in the signal peptide binding groove.

Results
- HDX reveals the dimer interface for the solution protein, which is more biochemically relevant than the many and sometimes contradictory solid-state structures determined by X-ray crystalllography.
- HDX also reveals an allosteric effect whereby the prepore binding domain becomes more open in the monomer than in the dimer, providing insight on the active form of SecA during in vivo protein translocation.

Overview

Methods
- **Protein expression:**
  - Full-length wild-type E. coli SecA without tags was expressed and purified as described previously (3).
- **HDX-MS analysis:**
  - Control dimer/monomer ratio: shift dimer/monomer equilibrium by changing KCl concentration (100 mM: dimer; 500 mM: monomer).
  - Conduct HDX-dilute SecA stock solution 1:5 with D2O buffer to incubate at 25 °C for a predetermined time (15, 30, 60, 150, 450, and 900 min) by quenching to 20 °C and 6 °C to 0 °C with pepfalin or pepfalin XIII.
  - Analyze by LC/MS: capture digested peptides on C8 trap column, desalt for 5 min, separate on a reversed-phase C18 column, detect by MS (Orbitrap).
- **Data Analysis:**
  - Use MassMatrix for peptide list identification to calculate D% with HDX workbench.

Figure 1. a. Crystal structure of SecA from E. coli (2SF8), different domains are represented by colors; b. Residue boundaries of E. coli SecA domains.

Figure 2. Dynamics of SecA monomer. The D% for all peptides at each HDX time point was plotted, as maps in Figure 3 show examples from various regions).

Results and Discussion

1. HDX dynamic map of SecA monomer
- D% from each HDX time point was mapped on the SecA monomer to compare HDX with the SecA crystal structure.
- Regions with defined secondary structures (α-helical or β-sheet) undergo slower HDX than unstructured regions or loops.

Table 1. Regions in SecA that show different D% incorporation between monomer and dimer. "Number of peptides" is number of overlapping peptides covering corresponding regions, all of which show consistent ΔD% values larger than 5 are considered large and otherwise small.

Table 2. Differential HDX Analysis of SecA monomer and dimer
To determine the dimer interface for SecA, HDX kinetics for various regions of SecA from solutions containing mainly monomer or dimer was measured (Figure 3 shows examples from various regions).
- a. Transducer helix from HSD
- b. Regions in PBD
- c. Two helix fingers from HSD
- d. Regions undergoing no structural change upon dimerization

Figure 3. Peptide-level HDX SecA. Comparison between monomer (black) and dimer (red) shows regions in (a), (b), and (c) that have greater ΔD% uptake in monomer than in dimer. (a), (b), and (c) are marked on Figure 4a.

Conclusions
- **Differential HDX identified a large interface of the SecA dimer in solution, involving mainly the HSD, which is consistent with analytical ultracentrifugation result (data not shown).**
- **Data also showed that SecA dimerization is linked to the open/closed conformational change in the preprotein binding domain (PBD), with the monomer more open than the dimer.**

References

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