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Supplemental Information

Can a Charged Surfactant Unfold an Uncharged Protein?

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Supporting Materials and Methods

MATERIALS AND METHODS

Materials: EXG:CBM and EXG:CBM^{QQQW} were produced as described (1). After lyophilization, the proteins were buffer exchanged into the appropriate buffer using either an Illustra NAP-5 (GE Healthcare) column (for CD, fluorescence and ITC experiments) or a Superdex-200 Increase 10/300GL (Amersham Biosciences) column (for SAXS experiments).

Equilibrium fluorescence: This was measured on an LS55 fluorimeter (Perkin Elmer). Samples contained ~3 µg/ml protein and were left to equilibrate in various concentrations of SDS for 20 hours before measurements. Stopped-flow rapid kinetics were carried out using 1:1 mixing of protein solution (final concentration 10 µg/ml) and SDS at the desired concentration on an SX20 rapid reaction microanalyzer (Applied Photophysics). All measurements were made at 25 ^{o}C in 10 mM NaP_i, pH 7.0.

Circular dichroism: Measurements were made on a J-810 spectropolarimeter (Jasco). All samples contained 0.25 mg/ml protein. Samples used for equilibrium measurements were left to equilibrate for 2 hours, while samples used for determining slow kinetics were mixed directly into the CD cuvette right before the start of the measurements, giving a dead time of 20 sec. All measurements were made at 25 ^{o}C in 10 mM NaPi pH 7.0.

Isothermal titration experiments: A VP-ITC instrument (MicroCal) was used. A solution of 75 mM SDS in 10 mM NaP_i, pH 7.0 was titrated in 5 µl aliquots into the cell containing 0.5-1.25 mg/ml protein in 10 mM NaP_i, pH 7.0. A long period (1000 sec) between injections was necessary due to a slow return to the baseline in the first part of the titration. Binding stoichiometries at the different transition peaks were calculated as follows (2):

$$[SDS]_{tot} = [SDS]_{aq} + N\,[Protein] \qquad (1)$$

where $[SDS]_{tot}$ and $[SDS]_{aq}$ are the total and non-bound concentration of SDS in the cell respectively and N is the number of SDS molecules bound to each protein at a given transition peak.

SAXS experiments: 2 mg/mL protein was used in a 10 mM NaPi buffer, pH 7.0. SAXS spectra of the proteins were measured in the absence of SDS and with four different SDS concentrations corresponding to the four transitions obtained in the ITC experiment. These SDS concentrations were 1.4, 4.51, 7.69 and 11.00 mM for EXG:CBM and 1.1, 4.38, 7.23 and 11.07 mM for EXG:CBM^{QQQW}. All samples and a 10 mM NaPi background sample were measured for 30 min at 20°C. The SAXS measurements were performed on Aarhus University's optimized NanoSTAR SAXS from Bruker AXS with a liquid Gallium jet X-ray source using a wavelength of 1.34 Å (3). The appropriate background spectra measured the same day were subtracted. For this and for the conversion to absolute scale, the SUPERSAXS software package (C.L.P. Oliveira and J.S.Pedersen, unpublished) was used with water as standard. All SAXS spectra are plotted as intensities I(q) as a function of the magnitude of scattering vector $q = (4\pi/\lambda)\sin\theta$, where θ is defined as half the scattering angle and λ is the X-ray wavelength (1.54Å).

SAXS analysis: Samples with SDS concentrations corresponding to the second to fourth binding point determined by ITC were analyzed using an SDS-protein core-shell model, in which SDS forms a central micelle surrounded by a shell of unfolded protein (4). For protein samples without SDS, the spectra were fitted with the solved structure of EXG:CBM (5), with the concentration of the protein as input parameter and with a random-walk structure factor describing the aggregation of the protein as a fitting parameter. Also, specific dimers were generated from the PDB files, and fitted to the spectra together with the monomer in a linear combination. Samples with SDS concentrations corresponding to the first ITC binding point were analyzed using a model of two associated proteins with a bridging layer of SDS.

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