SUPPLEMENTARY INFORMATION

Effect of Surfactant Hydrophobicity on the Pathway for Unfolding of Ubiquitin.

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MATERIALS AND METHODS.

Chemicals. Ubiquitin from bovine erythrocytes (all experiments with the same lot #075K7405), Tris-NH_2, Glycine, sodium salts of decyl sulfate, dodecyl sulfate, and tetradecyl sulfate, N,N-dimethylformamide, and HEPBS were purchased from Sigma-Aldrich. Dialysis microtubes (Slide-A-Lyzer MINI, MWCO 3.5k) were purchased from Pierce and rinsed several times with ultra-pure water prior to use. Surfactants were recrystallized three times from hot ethanol, and the absence of any alcohol precursor (dodecanol) was checked by NMR. Tris-glycine buffer (25 mM Tris, 192 mM glycine) was prepared from salts purchased from Aldrich and were filtered through a 0.22-μm membrane prior to use. Preparation of UBI in solutions of surfactants by dialysis was carried out as described in a previous study.[1]

Capillary Electrophoresis (CE): We used a Beckman Coulter P/ACE electrophoresis apparatus, with a capillary length of 60.2 cm (50 cm to the detector), and composed of fused silica (inner diameter of 50 μm; Polymicro Technologies TSP050375, Part#2000017, lot LHQ02A). Buffers used were composed of Tris-glycine (pH 8.4) containing concentrations of SDS (from 0 to 10 mM). CE was performed at 25 °C in a capillary with total length of 60.2 cm, a length from injection to the detector of 50.0 cm and with an applied voltage of 30 kV from 10 minutes. The capillary was equilibrated before each electrophoresis by a sequential rinsing with i) methanol, ii) HCl (1M), iii) NaOH (0.1 M), iv) ultrapure water (Millipore Advantage Ultrapure Water System, conductivity of 18.2 MΩ.cm and total organic content not exceeding 4 ppb), and v) the running buffer for the electrophoresis (3 minutes at 20 psi prior to each injections of protein). The solutions containing UBI·SC_nS complexes (~50 μM; we used an extinction coefficient for UBI of □(280) = 1490 L/(M·cm)^2 were injected in the capillary at 0.5 psi for 20 s. Samples were injected at a temperature of 4 °C and the temperature of the capillary was maintained at 25 °C during electrophoresis. Each series of experiments was carried out with the same capillary, equilibrated after each set of 20 runs.

The mobility of a UBI-SDS complex was calculated according to Eq. 1 where μ is the mobility of the migrating species (in cm^2 kV^{-1} min^{-1}), L_T is the total length of the capillary, L_D the length up to the detector, V the applied voltage, t_{eof} the time of the neutral marker, and t the time of the migrating species:

$$\mu = \frac{L_D \cdot L_T}{V}\left(\frac{1}{t_{eof}} - \frac{1}{t}\right) \qquad (1)$$

Correcting values of electrophoretic mobility of UBI-(SC_nS) complexes for changes in mass and hydrodynamic drag. A correction was introduced into the calculation of the number of surfactant molecules that bind UBI, according to the electrophoretic mobility as measured by capillary electrophoresis. The correction was performed by measuring the electrophoretic

mobility (μ_+) of each rung of a 4-sulfophenylisothiocyanate charge ladder (labels (+), Figure 2), and correcting each value of mobility for the increase in mass associated with thiocarbamylation (M_X = 214.2 Da) and the added mass resulting from the association of p molecules of SC_nS. The resulting change of charge $\Delta Z \sim -2$ with each thiocarbamylation provides a greater range for calibration of the UBI mobilities than does acetylation with acetic anhydride ($\Delta Z \sim -1$). We used Eq. 2, derived previously [1], where M_{C_nS} is the mass of the surfactant, and □ a constant generally equal to 2/3 for globular proteins[3]; M_{SC_nS} = 237.4 Da for n = 10, 265.4 Da for n = 12, and 293.4 Da for n = 14; the sodium ion is the counter-ion and does not contribute to the difference in mass.

$$\mu_{UBI-(SC_nS)_p} = \mu_+ \cdot \left(\frac{M_{UBI} + \frac{p}{2} \cdot M_X}{M_{UBI} + p \cdot M_{SC_nS}} \right)^{\alpha} \qquad (2)$$

Mass-calibrated mobilities for all complexes formed between UBI and $SC_{10}S$ (●), $SC_{12}S$ (○), and $SC_{14}S$ (□) were then fitted to a log-normal equation. The log-normal form, (equation in Figure 2a) has coefficients μ_0 = 25.013 cm^2 kV^{-1} min^{-1}, A = -21.38 cm^2 kV^{-1} min^{-1}, p_0 = 1.2513, while the scaling coefficients B deviate by less than 1.7% between the three curves.

The fitting procedure allowed us to use the mobility of a UBI·$(SC_nS)_p$ complex to approximate the number p of surfactant molecules bound to each protein. We can draw "rulers" to determine the stoichiometry p of complexes based on mobility (Figure 1, 2b). We used rulers obtained for each surfactant from calibration of charge ladders and equation 2 (rulers were superimposed on CE electropherograms in Figure 1).

The approximate stoichiometry that can be inferred from the "rule of thumb" of 1.4 gram of SDS that is bound per gram of protein in fully denatured form, as in typical SDS-PAGE separation [4] (p = 42), was found to be in agreement with the mobility of denatured (final)

complex of ubiquitin.[1]

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