Structural analysis of the ER-60/CNX complex using inverse contrast-matching small-angle neutron scattering

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ER-60, an oxidative protein folding enzyme that belongs to the PDI family, is a multi-domain protein consisting of four thioredoxin-like domains, **a** and **a'** domains with catalytically active cysteine pairs and chaperone association **b** and **b'** domains in the order **a-b-b-b'-a'**. ER-60 interacts with the arm-like P domain of calnexin (CNX), a chaperone that recognizes glycans, through the binding site for glycoprotein folding. However, the structure of ER-60 in complex with CNX has not been clarified, and there are many unsolved details of the complex regarding the mechanism of function in the oxidative folding. Using SAXS measurement, we have previously shown that the gyration radii R_g of ER-60, not in complex with CNX, differs depending on the oxidized or reduced form of the active cysteine pair in solution and the oxidized ER-60 takes an expanded shape, while the reduced ER-60 takes a compact shape [1]. However, it is not certain how the structure of ER-60 with CNX binding changes depending on the state of the active center. Inverse Contrast Matching Small-Angle Neutron Scattering (iCM-SANS) [2], which takes advantage of the large difference in neutron scattering length between hydrogen and deuterium, is useful for observing the structures of specific molecules in such complexes.

First, we prepared hydrogenated wild type (WT) ER-60 and 75% deuterated **a** domain fragment of ER-60 with the *E. coli* expression system. To prepare 75% deuterated recombinant protein, *E. coli* was cultured in 75% deuterated M9 medium. Recombinant proteins were purified. The deuteration rate of 75% deuterated protein was calculated by mass spectrometry with MALDI-TOF MS using a previously reported [3]. The deuteration rate of 75% deuterated **a** domain was calculated to be 73.6% from the increase in mass due to deuteration of un-exchangeable hydrogen obtained from MALDI-TOF MS (Fig. 1).

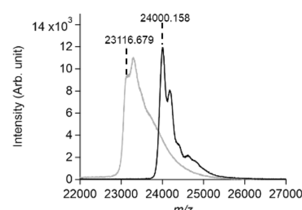

Fig. 1. Mass spectra of hydrogenated (gray line) and 75% deuterated (black line) **a** domain of ER-60.

1mg/mL 75% deuterated **a** domain and hydrogenated WT of ER-60 were measured using SANS in 100% D_2O buffer (20 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 1 mM $CaCl_2$) at 25 °C. As expected, 75% deuterated **a** domain was scatteringly invisible due to contrast matching (Fig. 2A), while hydrogenated WT was observed (Fig. 2B). In future, we will examine appropriate protein concentrations and instrument settings for iCM-SANS measurements of ER-60 and CNX complexes.

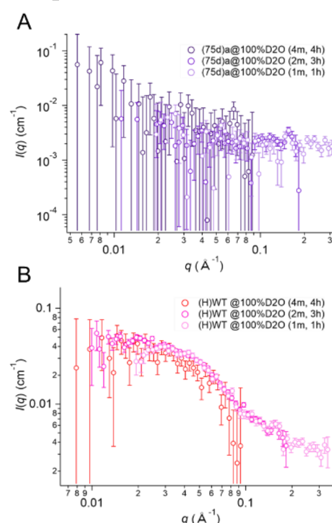

Fig. 2. SANS profiles of (A) 75% deuterated **a** domain and (B) hydrogenated WT of ER-60.

[1] A. Okuda *et al.*, Sci Rep., **11,** 5655 (2021).
[2] M. Sugiyama *et al.*, J. Appl. Crystallogr., **47,** 430–435 (2014).
[3] A. Okuda *et al.*, Biophys Physicobiol., **18,** 16-27 (2021).