Comment on "N-terminal Protein Tail Acts as Aggregation Protective Entropic Bristles: The SUMO Case"

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Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL - Center for Infection and Immunity of Lille, F-59000 Lille, France.

In a paper published in 2014,[1] Grana-Montes, R. et al. studied the secondary structure and aggregation of human small ubiquitin-like modifier 2 (SUMO-2) and proteins derived thereof. Like ubiquitin, SUMO are small proteins that are enzymatically attached to the target proteins through an isopeptidic bond linking the C-terminal glycine residue of SUMO to the side-chain amino group of surface exposed lysine residues. SUMO-2 is one of the five human SUMO paralogs identified so far and is frequently studied for being constitutively expressed in all eukaryotic cells, like SUMO-1 and SUMO-3.[2,3]

The study of the role of SUMO-2 tail, i.e. the first 14 amino acid residues (Fig. 1A), in protecting SUMO-2 from aggregation led the authors to analyse SUMO-2 and its truncated variant ΔNt-SUMO-2 starting at residue 15 by circular dichroism (CD) in the far-UV region using 50 mM phosphate buffer (pH 7). The authors found that the CD spectra of the two proteins are “essentially identical” (Fig. 2A in their article,[1] which presents the ellipticity Θ in mdeg between 195 and 270 nm at 298 K).

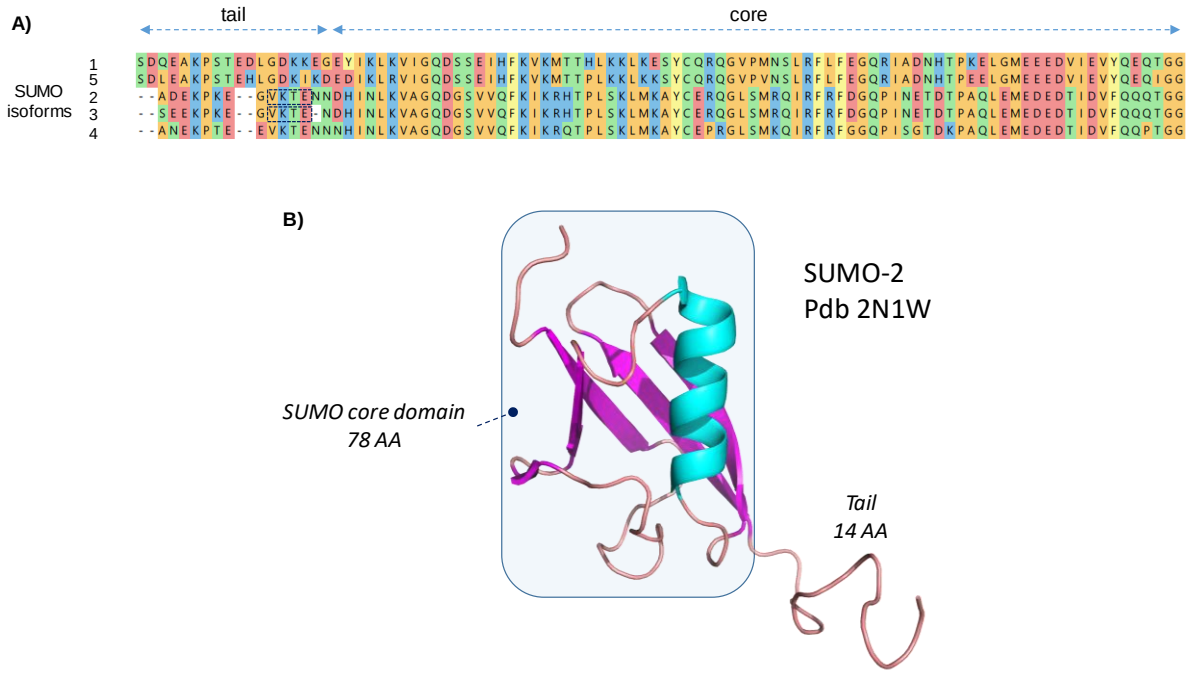

Figure 1. A) Protein sequences from human SUMO isoforms. Color background of amino acids corresponds to the chemical nature of the side chain (aromatic – yellow, acidic – red, basic – blue, nonpolar – orange, polar – green). See ref [4] for more details. B) NMR structure of SUMO-2 showing the N-terminal tail and the core domain (pdb 2N1W[5]).

Our recent studies[4,6,7] on the stability and biochemical properties of SUMO proteins led us also to compare SUMO-2 (92 AA) and ΔNt-SUMO-2 (78 AA) by the same technique. The proteins were produced by chemical synthesis using the *bis*(2-sulfanylethyl)amido-mediated ligation of two unprotected peptide segments in water.[8-12] Their homogeneity and identity was verified by UPLC-MS.[4] The far-UV CD spectrum of SUMO-2 protein was similar to a commercially available SUMO-2 recombinant product. Synthetic SUMO-2 protein was also analyzed by SDS-PAGE using Coomassie blue R250 staining, by Western-blot using an anti-SUMO2/3 antibody and its functionality was verified by performing an enzymatic conjugation assay using RanGAP as target protein. Moreover, the assembly method was validated by the production of various functional conjugates including SUMO-2/3 dimers.[4,6] Our data suggest that the far-UV CD spectra of SUMO-2 and ΔNt-SUMO-2 are different when plotting the mean residue ellipticity $[\Theta]_{MRW}$ against the wavelength between 190 and 260 nm at 298 K.[4] Note that the buffer used in our previous work (10 mM sodium phosphate buffer, pH 7.2)[4] or in the studies reported in this Comment (see later) for solubilizing the proteins is different from those used by Grana-Montes, R. et al. in their study.

Intrigued by this discrepancy, novel batches of SUMO-2 and ΔNt-SUMO-2 proteins were produced. We produced also the 14 AA peptide corresponding to the tail. During our experiments, we noticed that ΔNt-SUMO-2 had a limited solubility in phosphate buffer, which could be improved significantly by adding 50 mM NaCl. To facilitate the CD analysis of the proteins, we used instead a sodium phosphate/NaF buffer that has the advantage of being more transparent to UV light below 200 nm and thus more appropriate for providing high quality far-UV CD spectra.[13] SUMO-2, ΔNt-SUMO-2 and the tail peptide were analyzed by CD by performing four independent experiments. The results expressed as $[\Theta]_{MRW}$ (mean $\pm$ 95% confidence limits) against the wavelength between 185 and 260 nm are presented in Fig. 2. With these data, we confirm that the far-UV CD spectra of SUMO-2 and ΔNt-SUMO-2 are significantly different. The alpha-helical content (mean $\pm$ 95% confidence limits) of SUMO-2 (15.7 $\pm$ 0.47%, n = 4) calculated using the empirical equation of Greenfield & Fasman is significantly lower than those measured for ΔNt-SUMO-2 (17.5 $\pm$ 0.23%, n= 4), a result which is in agreement with the data obtained after deconvoluting CD spectra using Dichroweb online server (see Supplementary Information). Moreover, the CD spectrum of SUMO-2 shows a minimum at 205 nm, which is found at

208 nm in the spectrum of ΔNt-SUMO-2 protein. The CD spectrum of the tail peptide shows that it is largely in a random coil conformation with a minimum at 198 nm.

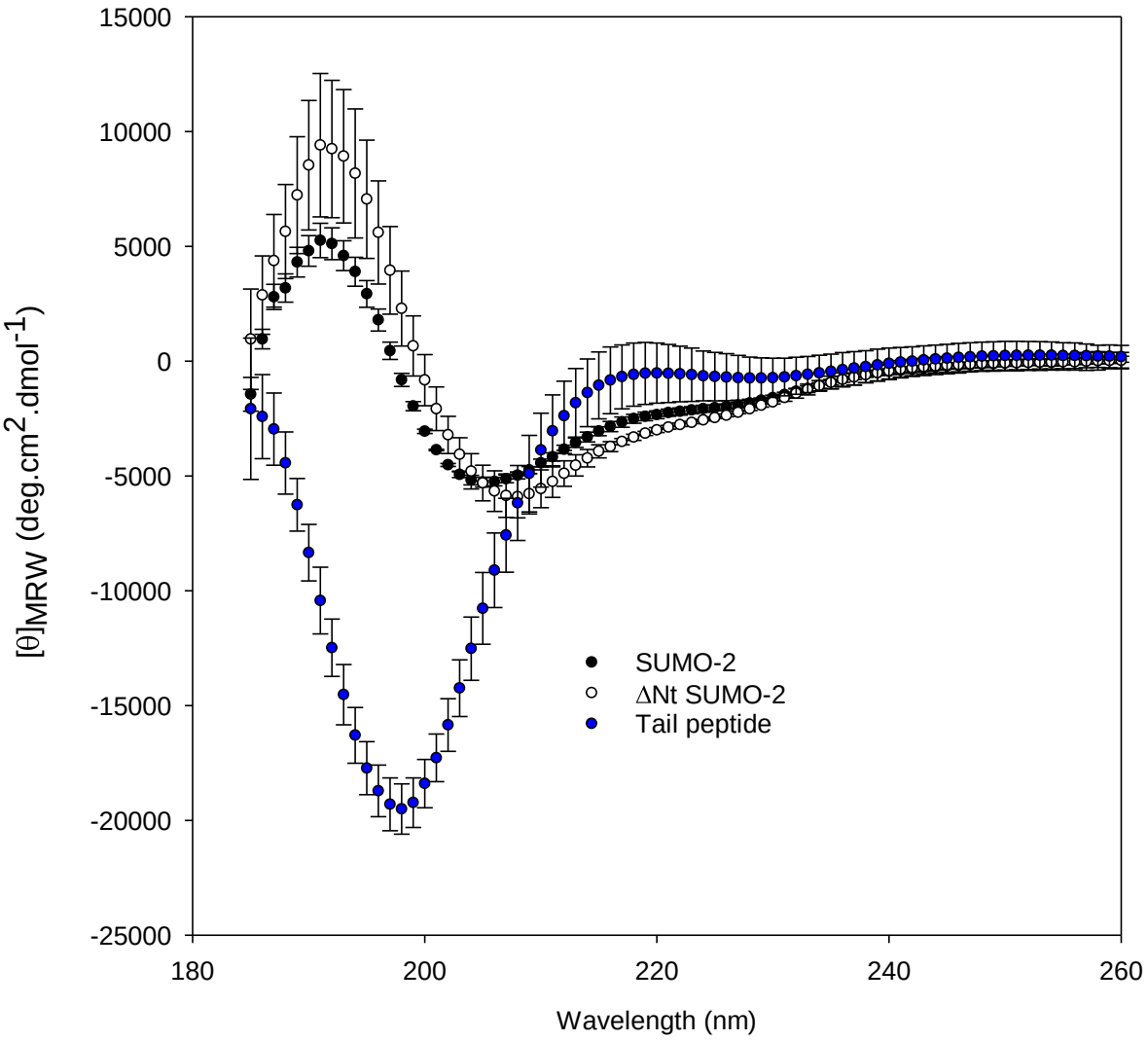

Figure 2. CD analysis of synthetic SUMO-2, ΔNt-SUMO-2 and tail peptide. Conditions: 10 mM phosphate buffer/50 mM NaF pH 7.2, 25 °C. The samples were carefully degassed during 30 min with argon and immediately transferred under argon into the CD cuvette. Protein concentration was 10-20 µM for SUMO-2 and ΔNt-SUMO-2 (see Supplementary Information). Tail peptide concentration was 100-230 µM (see Supplementary Information). The results expressed as $[\Theta]_{MRW}$ (mean ± 95% confidence limits) where obtained by performing four independent analyses. Full experimental details, CD data and the data obtained after the deconvolution of CD spectra using Dichroweb online server can be found in the Supplementary Information files.

As largely discussed in the literature, the tail of SUMO-2 protein is poorly structured (pdb 2AWT,[14] pdb 2N1W see Fig. 1B,[5] pdb 2N9E[15]). In contrast, the core domain of SUMO-2 which corresponds to the sequence of ΔNt-SUMO-2 adopts a typical ubiquitin fold, with an alpha-helix packed against a four-stranded beta-sheet (Fig. 1B). The far-UV CD spectrum of a protein can be represented by a linear combination of the spectra of its secondary structural elements, which include the disordered parts of the protein.[16] Therefore, the CD spectra of SUMO-2 and ΔNt-SUMO-2 are expected to be different in the light of the structural studies available for SUMO-2. Logically, the presence of the disordered tail in SUMO-2 lowers the alpha-helical content of the protein ($15.7 \pm 0.47\%$) compared to ΔNt-SUMO-2 ($17.5 \pm 0.23\%$) and also explains the shift of 3 nm in the position of the minimum around 208 nm.

In conclusion, the far-UV CD spectra of SUMO-2 and ΔNt-SUMO-2 are significantly different. We provide a robust protocol for the far-UV CD analysis of these proteins as well as all the data as a Supplementary Excel file for those interested in the study of SUMO protein conformation.

Acknowledgment

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Supplementary Information Available

Experimental procedure for CD analyses can be found in the Supplementary PDF file. CD data and deconvolution results can be found in the Supplementary Excel file. Data source files (.txt format) for all analyses are also provided in a compressed folder.

The Supplementary Information is available free of charge on the ACS Publications website at DOI: ##########.

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