NMR study of small protein asymmetric dimer

Dmitrii A. Luzik[1], Irene Ceccolini[2], Boris B. Kharkov[1], Alexandra D. Levkina[1], Ivan S. Podkorytov[1], Lewis E. Kay[3,4,5], Nikolai R. Skrynnikov[1,6]

[1]Laboratory of Biomolecular NMR, St. Petersburg State University, St. Petersburg 199034, Russia
[2]Department of Structural and Computational Biology, University of Vienna, Max F. Perutz Laboratories Vienna Biocenter Campus 5, 1030 Vienna, Austria
[3]Department of Molecular Genetics, University of Toronto, Toronto, ON M5S 1A8, Canada
[4]Department of Chemistry, University of Toronto, Toronto, ON M5S 3H6, Canada
[5]Department of Biochemistry, University of Toronto, Toronto, ON M5S 1A8, Canada 6 Program in Molecular Medicine, Hospital for Sick Children Research Institute, Toronto, ON M5G 0A4, Canada
[6]Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States

LCB3 is a de novo designed small three-helix bundle protein with a molecular weight of ~7.7 kDa [1], demonstrating capability to efficiently block S protein receptor biding domain of the coronavirus SARS-CoV2 binding to human ACE2. In order to improve LCB3 affinity for mutant variants of target protein, we proposed a mini-protein mutant with the point replacement of threonine 10 to tyrosine - LCB3 T10Y.

When assessing the structural properties of LCB3 T10Y using heteronuclear NMR spectroscopy, we found that the ^{1}H-^{15}N HSQC spectrum of this protein contains a set of peaks almost twice as large as expected from the amino acid sequence. This observation led us to propose that LCB3 T10Y is an asymmetric homodimer undergoing a slow conformational or monomer-dimer exchange. We performed PFG measurement of protein diffusion coefficient and relaxation rates (T_1 and $T_1\rho$) for this protein in order to estimate the size of the molecule.

Results of these measurements confirmed our dimer hypothesis, showing narrow distribution of $R_1\rho$ rates corresponding to approximate protein molecular weight ~14.2 kDa (while theoretical LCB3 T10Y monomer molecular weight is 7.8 kDa) and hydrodynamic radius [2] about 22.3 Å, which is also characteristic for the dimeric form. Finally, we measured dimerization K_d using isothermal titration calorimetry and found it to be equal to be 47.9±7.7 μM.

In order to asses structural features of LCB3 T10Y at more detailed level we acquired a set of 3D-NMR spectra for double-labeled (^{13}C, ^{15}N) samples and assigned the most part of backbone and sidechain signals. Chemical shifts-based TALOS+ secondary structure prediction shows that both subunits in dimer retain monomer-like three-helix structure. In the future we plan obtain and validate structural model of the dimer using docking and/or molecular dynamics guided by spatial renstraints (solvent PRE and NOE data) and chemical shifts from NMR spectroscopy. We also plan to crystallize the LCB3 T10Y sample followed by obtaining a high-resolution structure using X-ray diffraction analysis. We hope that our study will contribute to development of methodology of NMR-based studies of such interesting systems and shed some light on mechanisms of protein asymmetric dimers formation.

Acknowledgments

The authors acknowledge Saint-Petersburg State University for a research project 15.61.2221.2013

References

1. Cao, L., Goreshnik, I., Coventry, B., Case, J. B., Miller, L., Kozodoy, L., Chen, R. E., Carter, L., Walls, A. C., Park, Y.-J., Strauch, E.-M., Stewart, L., Diamond, M. S., Veesler, D., & Baker, D. (2020). De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. In Science (Vol. 370, Issue 6515, pp. 426–431). American Association for the Advancement of Science (AAAS). https://doi.org/10.1126/science.abd9909
2. Wilkins, D. K., Grimshaw, S. B., Receveur, V., Dobson, C. M., Jones, J. A., & Smith, L. J. (1999). Hydrodynamic Radii of Native and Denatured Proteins Measured by Pulse Field Gradient NMR Techniques. In Biochemistry (Vol. 38, Issue 50, pp. 16424–16431). American Chemical Society (ACS). https://doi.org/10.1021/bi991765q