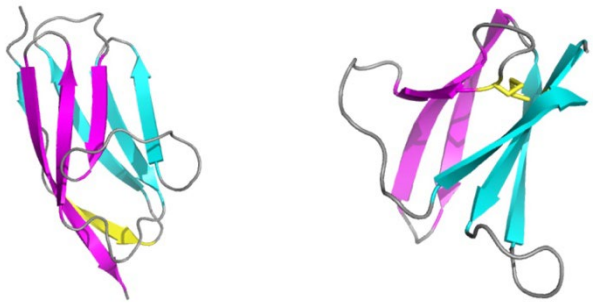
Do similar folding pathways mean similar folds ? A comparative High-Hydrostatic-Pressure NMR folding study of ß-sandwich proteins.

Christian ROUMESTAND (Montpellier University, France),

Abstract

If we know from Anfinsen laws that the 3D structure of a protein is encoded in its sequence, the paths taken by the polypeptide to fold into a well-defined native structure are far to be well understood. This issue is particularly important in the case of proteins having a similar 3D fold, but very divergent sequences. For instance, all the proteins from the Immunoglobulin Super Family (IgSF) are built on a common fold: a 7-stranded ß-sandwich called Ig-fold, but they differ in their distribution, biological role, and also in their amino acid composition. Similarly, MAX effectors are a family of proteins widely distributed in the phytopathogen fungus *Magnaporthe oryzae* that share a conserved common 6-stranded ß-sandwich fold, despite low sequence identity. These effectors constitute the molecular arsenal for the fungus infection and contribute to the pathogenicity of the plant pathogen.



The ß-sandwich 3D Structure of Titin I27 (left) and AVR-Pib (right)

In the present study, we compared the folding pathways of two Ig-like folds (I27 module from Titin, a multi-modular protein found in vertebrate striated muscle, and ED3, a domain from the Dengue virus envelope E-protein), and 3 MAX effectors (AVR-Pia, AVR-Pib and MAX60). We used High-Hydrostatic-Pressure NMR (HHP-NMR), a technique particularly well suited to decipher protein folding landscapes [1,3], allowing the identification of folding intermediates that could be otherwise rubbed out by harsher method as chemical denaturation, for instance.

Steady-state HP-NMR experiments show that the unfolding of both Ig-like modules start with the disruption of connections between the N- and C-termini, yielding a similar folding intermediate, even though real-time HP-NMR kinetics studies suggest a markedly more hydrated transition state ensemble (TSE) for ED3 than for I27 [4,5]. In the case of MAX effectors, we found that AVR-Pia and AVR-Pib display a similar folding intermediate formed by the ß-sheet ß3ß4 [6]. On the other hand, MAX60 exhibits a different folding intermediate, consisting in its additional C-terminal helical extension and the ß-sheet ß1ß6 [7]. Thus, proteins with similar topology can present similar folding pathways, but in the frame of a similar structural context. In the case of MAX60, the C-terminal extension that makes close contacts with the protein core is able to change the folding pathway of the ß-sandwich.

References

[1] Roche J, Royer CA, Roumestand C. (2017) Prog. Nucl. Magn. Reson. Spectrosc. 102-103, 15-31.
[2] Roche J, Royer CA, Roumestand C. (2019). Methods Enzymol. 614:293.
[3] Dubois C, Herrada I, Barthe P, Roumestand C. (2020) Molecules. 25, 5551.
[4] Herrada I, Barthe P, Vanheusden M, DeGuillen K, Mammri L, Delbecq S, Rico F, Roumestand C. (2020), Biophys. J. 115: 341-352.
[5] Saotome T, Doret M, Kulkarni M, Yang YS, Barthe P, Kuroda Y, Roumestand C. (2019), Biomolecules 9: pli: E309.
[6] Dubois C, Lahfa M, Pissarra J, de Guillen K, Barthe P, Kroj T, Roumestand C, Padilla A. (2022). Int J Mol Sci 23, 8469.
[7] Lahfa M, Mouhand A, de Guillen K, Barthe P, Kroj T, Padilla A, Roumestand C. (2023) Molecules 28, 6068.