Hinge Peptide and Intersubunit Interface in Domain Swapping

Filomena Sica[1,2], Delia Picone[1], Antonello Merlino[1], Anna Di Fiore[1], Carmine Ercole[1], Marisa Franzese[1], Lelio Mazzarella[1,2], *[1]Dipartimento di Chimica,Università di Napoli Federico II. [2]Istituto di Biostrutture e Bioimmagini, CNR.* E-mail: filosica@unina.it

The domain swapping is a phenomenon, which is observed in an increasing number of proteins. Among the swapped proteins, bovine seminal ribonuclease (BS-RNase) represents a unique example, as the process occurs in the native mixture between two dimeric forms, MxM (swapped) and M=M (unswapped), held together by the additional constraints of two inter-chain disulphide bridges. This peculiarity offers the opportunity to study the effects of selected mutations at the O-interface on the M=M / MxM equilibrium, within a substantially invariant quaternary assembly. Two variants, having Pro 19 (P19A) or the whole sequence of the hinge peptide 16-22 replaced by the corresponding residues of RNase A (BS-hinge-A), show equilibrium and kinetic parameters of the swapping similar to those of the parent protein. On the contrary, mutation of L28 (L28Q, P19A/L28Q) significantly affects the swapping processes. The X-ray structures of the MxM forms of P19A and BS-hinge-A, and the MxM and M=M forms of the double mutant P19A/L28Q have been determined. The structural effects of the mutations are discussed on the basis of the MxM/M=M equilibrium data measured in solution. The relative insensitivity of the swapping tendency to the substitutions in the hinge region, and in particular to the replacement P19A, contrasts with the results obtained for other swapped proteins and can be rationalized in terms of the unique features of the seminal enzyme. On the other hand, the substitution in position 28 points to a crucial role of the interface residues in the swapping of BS-RNase.

Keywords: swapping , ribonuclease mutations, structural analysis