Design and gene construction of Octarellin IV

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Octarellins are *de novo* designed proteins which are based on the α/β-barrel scaffold. An iterative procedure has been used to design these artificial proteins. Each step aiming at the improvement of the folding and stability of the protein. The main purpose of the design of the second generation of Octarellin was to improve the solubility of the poorly soluble Octarellin I[1] and to introduce in the artificial structure the β-sheet barrel 4-fold symmetry of the natural scaffold. This led to a higher solubility of Octarellin II and to a better stability of Octarellin III when compared to the previous generation[2].

An homology modeling study has revealed two main problems which potentially could destabilize the protein or prevent it from folding correctly. The β/α turns appears too long and too hydrophobic which probably favors protein aggregation and also, because two lysine side chains appear to point toward each other, the α-helices tend to repulse each other.

For the next generation of Octarellin (Octarellin IV), these two problems have been dealt with, by the design of a new β/α turns and by the replacement of one of the above mentioned lysine by a glutamic acid residue on the even α-helix. In addition, several minor substitutions have also been introduced, when compared to Octarellin III.

A nucleotidic sequence has been deduce from this amino acid sequence taking into account the preferred codon usage in *E. coli*. In order to introduce intrinsic spectroscopic probes, the methionine residue of the even β-strand of the second and fourth subunit has been replaced by a tyrosine and a tryptophan residue respectively. For the cloning strategy, it was also necessary to introduce on each side of the subunits, two restriction sites with compatible cohesive ends.

The nucleotidic sequences coding for the different subunits have been constructed by recursive PCR and fused into the Octarellin IV gene using a strategy similar to the one developed for the previous generation of Octarellin.

The expression and characterization of Octarellin IV will provide data which will be fed back into the iterative process that we are currently using to approach the protein *de novo* design problem.

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[1] Goraj *et al.* (1990) Protein Engng **3**, 259-266
[2] Houbrechts *et al.* (1995) Protein Engng **8**, 249-259