

Protein Engineering and Production

TOWARDS THE DESIGN AND SYNTHESIS OF AN ARTIFICIAL α/β BARREL PROTEIN.

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INTRODUCTION. Various algorithms predicting a protein 3D structure from its amino acid sequence are currently available. We have been working on several improvements of these algorithms. To assess (and further improve) our first round improvements, we engaged in the design and synthesis of an artificial α/β barrel protein.

EXPERIMENTAL APPROACH AND RESULTS. The α/β barrel proteins can be dissected into eight consecutive β strand- α helix units that we will refer to as "structural units" (Lebioda et al. (1)).

We first compared the amino acid sequences and structures of each structural unit of the known α/β barrel proteins. From all the constraints deduced from this analysis (size of the secondary structures, presence of hydrophobic diamond patterns, relative distributions of the amino acids, etc.), we proposed a first "consensus" peptide sequence for the structural unit.

To define a particular sequence from this "consensus sequence", we adapted the methods of Garnier (2) and Taylor and Thornton (3) and developed a program named "predipep". This program gives an optimal peptide sequence fitting average Garnier profiles (obtained here from the sequences of the natural α/β barrel proteins) and taking into account specific constraints (here, defined above).

We then deduced the nucleotide sequence and synthesized the oligonucleotide (99 mer) coding for the structural unit peptide. We finally inserted from 1 to 12 copies of this 99 mer in a expression vector of *E.coli* which was optimized for maximal expression of the artificial peptides. The 12 recombinant plasmids were introduced into *E.coli*. Eleven artificial peptides (from the dimer to the dodecamer) were expressed at high level and appeared quite stable in *E.coli*. In fact, they accumulated in the bacteria as inclusion bodies which were easily isolated by differential centrifugation providing so an already significant purification (Fig. 1).

Our level of expression allows us to hope for a mean yield of at least 5 mg of pure protein per liter of culture. We are currently in the process of testing the best conditions under which the inclusion bodies can be solubilized and the protein completely purified.

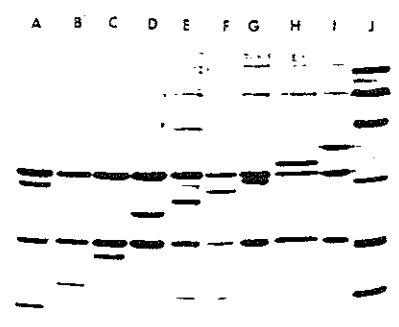


Fig. 1. SDS polyacrylamide gel electrophoresis of the proteins contained in inclusion bodies of induced *E.coli* transformed by plasmids coding for the 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, and 12- mer in lanes A to I respectively. Lane J contains molecular weight markers.

In a second type of approach, we are trying to verify whether a structural subunit can be looked at as an independant folding unit. This is done by expressing in *E.coli* various mutants, obtained by directed mutagenesis, of a natural α/β barrel protein: the triose phosphate isomerase of *E. coli* (4).

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