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AN EXPERIMENTAL APPROACH TO EXON SHUFFLING: MODULAR MUTAGENESIS OF BARNASE

Hiroshi Yanagawa, Masamichi Ishizaka, Kenji Yoshida, and Akira Sato, and Tamiyasu Shimamiya
Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan, FAX,Int.+427-24-6317.

Globular proteins are composed of several modules that are contiguous polypeptide segments of compact conformation(Go, 1983). Module boundaries often correspond to intron positions of eukaryotic genes(Go, 1981). Modules may closely correspond to exons in primordial genes. Most modern proteins arise through the mechanism of exon shuffling(Gilbert, 1978), which involves the recombination of whole exons. Exon shuffling is supported by assuming there to be physical or physicochemical reasons why coding sequence encodes a functional or structrual unit. For confirmation of this " exon shuffling hypothesis," structural and catalytic functions of disconnected modules of barnase were examined. Barnase, a ribonuclease from *Bacillus amyloliquefaciens,* is a small monomeric protein whose X-ray structure is known. The tertiary structure of barnase was decomposed into six modules (M1-M6) by compactness criterion using centripetal and extension profiles. We examined the conformation of these modules in aqueous solution. Among the six modules, only M1 and M5 aggregated to form filamentous helical structures and platelet structures, respectively (Yoshida *et al.*,1993). Circular dichroism spectra showed that M1 has a helical structure and M5 has a sheet structure. M1 and M5 contain the first of three helices and the 5 to 6 th of seven

sheets in the intact protein. M2, M3, and M6, all of which form a shallow but wide cavity for RNA binding in native barnase, bind to RNA and possess RNase activity (Yanagawa *et al.*,1993). However, M1 and M5, which support other modules from back side. The results strongly support the possibility that M1 and M5 are capable of spontaneously assembling with other modules to form a more active catalytic protein than modules alone.

Attempts are presently being made to produce novel protein molecules by shuffling of module fragments *in vitro*. A module was used here as a unit of shuffling. The total coding DNA sequence of barnase is 330 bp which was divided into DNA fragments corresponding six module fragments, 72, 84, 63, 45, 30, and 36 bp. We constructed 23 shuffled genes by multi-recombinant PCR. Protein mutants corresponding to the genes were successively expressed in *E. coli*. Their physicochemical properties were determined by exclusion chromatography, near- and far-UV circular dichroism, fluorescence and ^{1}H NMR spectroscopy. Among shuffled mutants, barsena, which was formed by permutation of M2 with M3, cooperatively unfolded with titration by urea, indicating the presence of specific tertiary interactions. Thermodynamic stability at pH 6.0 and 5 ℃ of wild-type barnase and barsena was 10.3 and 1.3 kcal/mol, respectively. Barsena is shown to have less ordered structure than wild-type barnase.

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