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SYNTHESIS OF A GENE CODING FOR VASOACTIVE INTESTINAL PEPTIDE (VIP)

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Summary: A solid phase phosphoramidite triester coupling approach was used for the synthesis of the 16 fragments of a gene coding for human/porcine VIP. The design, synthesis, purification, subcloning and sequencing of the gene will be described.

VIP is a highly basic, single chain polypeptide of twenty-eight amino acids which has been isolated from chicken, pig¹ and man². The porcine and human VIP are identical.³ Although originally isolated from gut, VIP has now been found widely distributed throughout the body with significant concentration in the brain and gut. Since a gene for VIP has not been isolated we have designed a gene sequence coding for porcine/human VIP. Due to the degeneracy of the genetic code, 2.8×10^{13} different nucleotide sequences correctly code for VIP. In arriving at one particular sequence we employed various discriminators such as codon frequencies for expression in yeast, placement of restriction endonuclease sites, ease of synthesis and elimination of regions of self-complementarity.

A double-stranded 99 base pair DNA sequence was then synthesized in 16 fragments. The chemical synthesis of the 16 fragments (varying in lengths from an 11-mer to a 14-mer) was achieved on a functionalized silica gel polymer support using the phosphoramidite triester coupling approach.⁴ The syntheses were carried out on a BioSearch SAM ONE synthesizer. Each fragment was fully deprotected, purified by acrylamide gel electrophoresis and sized. The nucleotide fragments were annealed and joined using DNA ligase. The gene coding for VIP was then subcloned in pBR322 and the DNA sequenced using Maxam-Gilbert sequencing procedure.

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