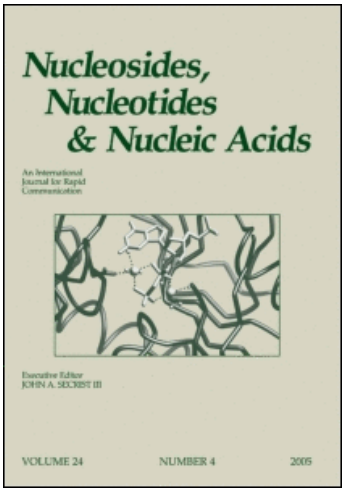

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USE OF LONG OLIGONUCLEOTIDES IN GENE SYNTHESIS: CHEMICAL SYNTHESIS AND CLONING OF A GENE FOR SALMON CALCITONIN

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Summary: The design, chemical synthesis and cloning of a gene for salmon calcitonin I-gly(33) consisting of two long oligodeoxyribonucleotides (109- and 117-mer) are described. Synthesizing both of the oligonucleotides on CPG supports with pore sizes of 500 or 1000 Å respectively, a superior performance of the 1000 Å material was observed.

Calcitonin is a hypocalcemic and hypophosphatemic peptide hormone involved in the regulation of the serum calcium level. Common characteristics of calcitonins from different species are a length of 32 amino acids, a disulfide bridge between cysteine residues 1 and 7 and a prolinamide at the carboxy terminus. Among the commercially available calcitonins (human, porcine, salmon, eel) salmon calcitonin is the most active. Calcitonin is used in the treatment of disorders in calcium metabolism (e.g. Morbus Paget, hypercalcemia, osteoporosis).

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   Sph I
                1                                   10
           Met Cys Ser Asn Leu Ser Thr Cys Val Leu Gly Lys Leu Ser Gln Glu Leu His
5'       C ATG TGC TCT AAC CTG TCG ACT TGC GTT CTT GGT AAG CTT TCT CAG GAA CTT CAT
3' GT ACG TAC ACG AGA TTG GAC AGC TGA ACG CAA GAA CCA TTC GAA AGA GTC CTT GAA GTA
              20                                  30                      Eco R I
  Lys Leu Gln Thr Tyr Pro Arg Thr Asn Thr Gly Ser Gly Thr Pro Gly Stp Stp
  AAA CTG CAG ACC TAT CCG CGC ACT AAT ACC GGC TCT GGT ACC CCT GGT TAA TAG       3'
  TTT GAC GTC TGG ATA GGC GCG TGA TTA TGG CCG AGA CCA TGG GGA CCA ATT ATC TTA A 5'
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In mammals calcitonin is produced in the C-cells of the tyroid from a precursor by posttranslational processing

of the combined carboxyterminal cleavage and amidation signal gly-lys-lys-arg. Therefore we designed a gene encoding the 32 amino acid sequence of salmon calcitonin I plus an additional codon for gly at the 3' end. Calcitonin-like activity is expected for salmon calcitonin-gly^{33}, which on the other hand can be converted enzymatically into the amidated form.

The total gene for salmon calcitonin-gly^{33} was designed from only two long oligonucleotides representing the coding (109-mer) and noncoding (117-mer) strands without need of an enzymatic ligation step. Furthermore codons for highly expressed proteins in E. coli were selected and regions of selfcomplementarity on the mRNA-level were avoided. A Sph I site was added at the 5' end of the gene, an Eco RI site at the 3' end and more than five restriction endonuclease sites were placed equidistantly in the gene to facilitate further modifications.

Syntheses were performed with Applied Biosystems Model 380 A and B synthesizers using ß-cyanoethylphosphoramidites. The two oligonucleotides were synthesized twice, first on a CPG support with a pore size of 500 Å in a 1 µmol scale (10-fold excess of phosphoramidites), second on a 1000 Å CPG support (Applied Biosystems) in a 0.2 µmol scale (25-fold excess of phosphoramidites). A superior performance of the 1000 Å CPG support was observed (e.g. the repetitive yield in detritylation (99,4%) was about 1% higher).

For cloning of the gene the oligonucleotides were hybridized, ligated into pUC 19 and transformed in E. coli. Subsequent dideoxy sequencing confirmed the nucleotide sequence of the gene. Salmon calcitonin-gly^{33} was expressed in E. coli as a fusion protein with a part of ß-galactosidase which after BrCN cleavage yielded the desired peptide.