SYNTHESIS OF FRAGMENTS OF THE SOMATOSTATIN GENE

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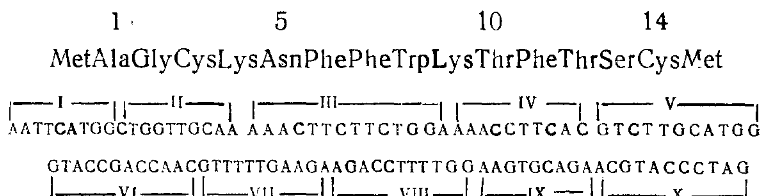
In a study of somatostatin we have performed the chemical synthesis of fragments (I-X) of the somatostatin gene.



The amino acid sequence of somatostatin was flanked by methionine residues for the subsequent cutting out of the functional hormone. When the gene was incorporated into a vector in the given orientation, half-sites of restrictases EcoRI and BamHI were provided at the ends of the gene.

The absence of stop codons after the sense sequence distinguished the nucleotide sequence shown from that published previously [1], and will permit the gene to be polymerized subsequently by the scheme proposed in [2] and, thereby, the final yield of hormone to be raised.

The nucleotide sequences in the translated and the complementary chains of the somatostatin gene (54 nucleotides in each chain) were divided into ten overlapping fragments. The dimensions of oligonucleotides (I-X) (from 9 to 14 units) were selected in such a way that the overlapping in complementary sections ensured an unambiguous course of the ligase linkage of the gene fragments.

Oligonucleotides (I-X) were synthesized by the block phosphotriester method in solution [3], the nucleotide chain being grown in the 3' → 5' direction. The starting materials for obtaining these blocks were N-benzoyl-3'-(cyanoethyl-p-chlorophenyl)-2'-deoxynucleotides [sic] (the OH components in internucleotide condensation) and N-benzoyl-5'-dimethoxytrityl-2'-deoxynucleoside 3'-(p-chlorophenyl phosphate)s (the P-components). 2,4,6-Triisopropylbenzenesulfontetrazolide [4] was used as the condensing agent. The condensation reaction was performed in absolute pyridine for 30-60 min. The yields range from 45 to 86%, depending on the length and composition of the blocks being obtained.

The products of internucleotide condensation ("dyads" and larger blocks) were isolated by chromatography on columns of silica gel (40-100 mesh, Chemapol; gradient elution with from 0 to 10% of methanol in chloroform at a pressure of 1-2 atm.).

After deblocking (25% NH_4OH, 60°C, 16 h; 80% AcOH, 20°C, 30 min), the desired oligonucleotides were isolated by chromatography on the ion-exchanger DEAE-Toyopearl-650S in a concentration gradient of NaCl in 7M urea at pH 7.4 and 3.5. For the final purification of the oligonucleotides (I-X) we used reversed-phase HPLC on a LiChrosorb C_{18} (4.6 × 250 mm) column (Altex) with a 6-30% gradient of methanol in 0.1 M ammonium acetate, 25 min. The homogeneity of the oligonucleotides isolated was checked with the aid of HPLC and electrophoresis in denaturing polyacrylamide gel after the introduction of a 5'-terminal label. The nucleotide sequence was confirmed by the Maxam-Gilbert method [5]. At the present time work is proceeding on the ligase assembly of the fragments obtained to form the complete somatostatin gene for subsequent passage to cloning and expression.

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CHARACTERIZATION OF TOXINS FROM VENOM OF *Vespa orientalis*

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The toxic properties of the venom of the hornet *Vespa orientalis* are due to the presence in it of lysophospholipase A_1, which possesses a presynaptic action [1], and of highly toxic phospholipase A_2 [2]. In the present paper the properties of these two toxins, designated as orientotoxin-I (ORT-I) and orientotoxin-II (ORT-II), are compared:

Parameters and acting agents	ORT-I	ORT-II
Toxicity, LD_{50}, mg/kg on intravenous injection	0.5	0.65
Molecular mass, kDa	16	15
Electrophoretic mobility, R_f	0.47	0.53
N-Terminal amino acid	phenylalanine	phenylalanine
C-Terminal amino acid	lysine	lysine
Concentration necessary for complete hemolysis, μg	0.075*	0.75*
Specific substrate	lysolecithin	lecithin
Optimum pH	7.5	8.5
Optimum temperature, °C	45	50
Optimum Ca^{2+} concentration, mM	5	10
Specific activity, units/mg	5480	29,400
Michaelis constant, mM	0.27	1.61
Inactivation constants, min^{-1}		
by trypsin	0.11	0.08
by p-bromophenacyl bromide	$3\cdot10^{-2}$	$2.9\cdot10^{-2}$
by bromomethyl adamantyl ketone	$2.1\cdot10^{-2}$	$2.0\cdot10^{-2}$
by heat treatment at 50°C	0.012	0.012
by urea (8 M)	$8.3\cdot10^{-2}$	$8.3\cdot10^{-2}$
Inactivation by detergents, mM		
sodium deoxycholate	11	16
sodium dodecyl sulfate	10	15
Number of peptides on hydrolysis		
by trypsin	13(2)	15(2)
by chymotrypsin	23(3)	22(3)
by glutamine proteinase	15(5)	16(5)

(continued on following page)

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