CHEMICAL SYNTHESIS OF TWO DEOXYRIBOPOLYNUCLEOTIDE FRAGMENTS CONTAINING THE NATURAL SEQUENCE OF T4 LYSOZYME GENE e.

S.A. Narang*, K. Itakura, C.P. Bahl and Y.Y. Wigfield

(Division of Biological Sciences,

National Research Council of Canada,

Ottawa, Canada K1S 5B6).

Received September 1, 1972

Summary

The chemical synthesis of two deoxyribopolynucleotide fragments, d-pApTpTpApApGpTpGpApTpGpG (dodeca-) and d-pApCpTpTpTpTpTpGpT (nona-), containing the natural sequence of T4 lysozyme gene e have been achieved by a new synthetic approach.

The strain eJ44J42 of phage T4, which carries proflavine induced mutation in the lysozyme gene e, produced a lysozyme protein differing from that of the wild-type strain by a sequence of five amino acids (1). On the basis of triplets proposed by Nirenberg (2) and Khorana (3), Streisinger and his coworkers (1) were able to assign a unique sequence of bases that would code for the five amino acid sequence in the wild-type lysozyme. By the addition and deletion of a single base, this sequence could be changed to one which would then code for the sequence of five amino acids in the double-mutant strain. Streisinger and coworkers (4) have also recently established another unique sequence of bases by studying the frameshift mutation in eJD10eJD11 strain of phage T4. These findings

Copyright © 1972 by Academic Press, Inc.
All rights of reproduction in any form reserved.

make it possible to write the actual sequence for a piece of T4 lysozyme gene e (5) (see Scheme). In this communication, we wish to report the chemical synthesis of these dodeca (I) and nonanucleotides (II) by a new chemical methodology developed in our laboratory. The synthesis of segments (I) and (II) is pursued as a preliminary step in the synthesis of the T4 lysozyme gene e: ligase-joining of (I) and (II) would be followed by elongation from 3'-hydroxyl end with DNA-polymerase in the presence of an appropriate complementary T4 DNA strand (6). This approach assumes that the dodeca-nucleotide segment is the nonrepetitive minimum recognition length (MRL) which is able to recognize its complementary sequence in a long DNA strand and forms a duplex (7).

Material and General Methods

All deoxymononucleoside 5'-phosphates (Calbiochem.), mesitylenesulfonyl chloride (MS) (Aldrich), Avicel-cellulose (0.1mm thickness) (Brinkman Inc.) Avicel-cellulose (1.0mm thickness) (Analtech.), Benzoylated DEAE-Cellulose (Schwarz Mann) and Sephadex (superfine grade) (Pharmacia) were purchased commercially. 2-Phenylmercaptoethanol (8) and Benzoylated DEAE-Sephadex (9) were prepared by the previously reported procedures.

All the protected and unprotected trinucleotides were prepared according to the new method described recently (10). The homogeneity and enzymatic characterization of oligonucleotides were carried on Avicel tlc plate (11).

Results and Discussion

Synthesis of Dodecanucleotide (I)

(a) BASE SEQUENCE OF A SPECIFIC REGION OF WILD-TYPE LYSOZYME GENE e OF T^4 DNA

5' --- ApCpApApApApGpTpCpCpApTpCpApCpTpTpApApTpGpCp --- 3'

3' --- TpGpTpTpTpTpTpCpApGpGpTpApGpTpGpApApTpTpApCpGp --- 5'

(II) (I)

(b) SYNTHESIS OF DODECANUCLEOTIDE (I)

PME-pApTpT-OH + pApApG-oAc (Ac Ac Ac) $\xrightarrow[(iv),(v)]{(i),(ii),(iii)}$ PME-pApTpTpApApG-OH

(III) (IV) (V)

(V) + pTpGpApTpGpG-oAc (Ac Ac Ac Ac) $\xrightarrow[(iv),(v)]{(i),(ii),(iii)}$ PME-pApTpTpApApGpTpGpApTpGpG

(VI) (VII)

↓ $NaIO_4$-2N NaOH(R.T., 90 mi

d-pApTpTpApApGpTpGpApTpGpG

(I)

(c) SYNTHESIS OF NONANUCLEOTIDE II

PME-pApCpT-OH + pTpTpTpTpGpT-oAc (DMM DMM Ac) $\xrightarrow[(iv),(v)]{(i),(ii),(iii)}$ PME-pApCpTpTpTpTpTpGpT

(VIII) (IX) (X)

↓ $NaIO_4$-2N NaOH(R.T. min)

d-pApCpTpTpTpTpTpGpT

(II)

(i) Mesitylenesulfonyl chloride
(ii) Conc. ammonium hydroxide, 50%/90 min.
(iii) Benzoylated DEAE-Column Chromatography
(iv) Sephadex G-75 (superfine) gel-filtration
(v) Preparative tlc

PME Stands for 2-phenylmercaptoethyl group
DMM Represents dimethylaminomethylene group

SCHEME

The chemical synthesis of (I) was achieved by a new synthetic approach outlined in the chart. Its basic principle is that the attachment of an aromatic protecting group to the 5'-phosphate of an oligonucleotide unit markedly increases its binding to benzoylated DEAE-Sephadex (9) or cellulose (12). On condensation of (III) with a second component, 3'-O-acetyl N-protected oligonucleoside 5'-phosphate (IV) containing non-aromatic protecting group, the aromatic group is only found in the unreacted starting material (III) and the product (V). Thus, when the reaction mixture is passed through a benzoylated DEAE-Cellulose column, (III) and (V) will be the only components of the reaction mixture retained by the BD-DEAE-Cellulose due to their affinity between their aromatic rings. All the other components, which lack the aromatic protecting groups such as unreacted (IV) and its pyrophosphate (a serious side product) will be eluted quickly with weak buffer. The component (III) and (V) can then be eluted by washing the column with strong buffer containing 50% ethyl alcohol. The desired product is subsequently separated from the starting material by gel-filtration on Sephadex G-75 (superfine) (13) and preparative tlc (14). For this synthetic approach, we have recently introduced 2-phenylmercaptoethanol, a novel phosphate protecting group (15) which is easily introduced and stable to conditions normally encountered in deoxyoligonucleotides synthesis. However, when oxidized to sulfoxide derivative with sodium metaperiodate, it is easily removed with 2N sodium hydroxide at room temperature in 90 min. It is noteworthy to state that the present synthesis of dodeca-nucleotide has been accomplished with no protection of the

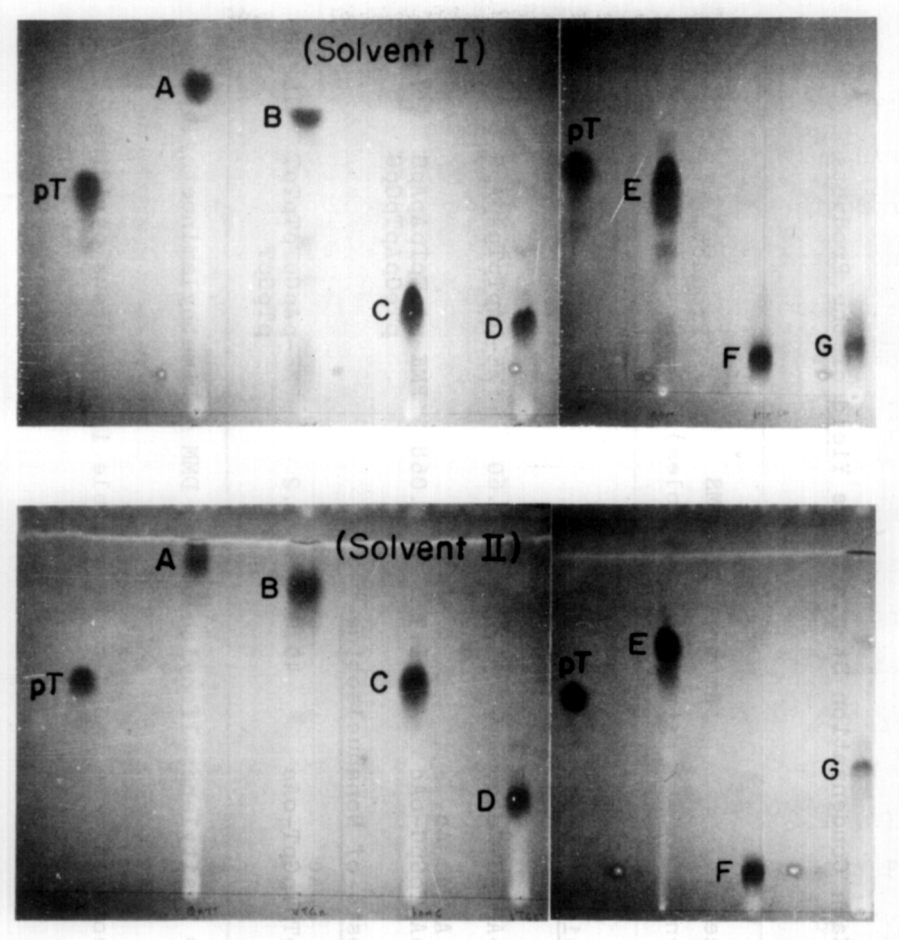

Figure 1. TLC on Avicel-Cellulose plates (0.1mm thickness), Solvent I, n-propanol:concentrated ammonium hydroxide and water (55:10:35 v/v); Solvent II, isobutyric acid:1M ammonium hydroxide:0.1M ETDA (100:60:1.6 v/v); A, PME-pApTpT; B, PME-pTpGpA; C, d-pApApG; D, d-pTpGpG; E, PME-pApTpTpApApG; F, d-pTpGpApTpGpG and G, PME-pApTpTpApApGpTpG pApTpGpG.

amino group in the case of adenine and guanine bases, a simplification of the present methodology, reported recently (16). The reaction conditions and yields of hexa-, nona- and dodecanucleotides are given in Table 1. The homogeneity of each intermediate was determined on Avicel tlc plate in two solvent systems as shown in Figure 1. Finally, each

Table I. Reaction Conditions of Each Condensation Step and the Yield of the Products

Phosphate Protected Component[a]	Amount (μmoles)	3'-O-Acetyl Component[a]	Amount (μmoles)	MS (mmoles)	Product	Yield (%)
		Synthesis of Dodecanucleotide				
PME-pApTpT-OH	25.0	d-pA^{Ac}pA^{Ac}pG^{Ac}oAc	30.0	0.60	PME-pApTpTpApApG	25-30
PME-pApTpTpApApG-OH	2.0	Ac Ac Ac Ac d-pTpGpApTpGpG-oAc	1.7	0.068	PME-pApTpTpApApG pTpGpApTpGpG	7-10
		Synthesis of Nonanucleotide				
DMM DMM PME-pApCpT-OH	60.0	Ac d-pTpTpTpTpGpT-oAc	40.0	1.2	PME-pApCpTpTpTpT pTpGpT	15-20

PME strands for 2-Phenylmercaptoethyl; MS = Mesitylenesulfonylchloride; DMM = Dimethylaminoethylene group.

[a]The tri-n-decylammonium salt of oligonucleotides were found to be soluble in anhydrous pyridine.

unphosphorylated oligonucleotide was found to be completely degraded with spleen and snake venom phosphodiesterase enzymes.

Synthesis of Nonanucleotide (II)

The synthesis of nonanucleotide (II) was accomplished by an alternative approach (see chart). It is mainly based upon a concept of condensation between the smaller unit of aromatic protected oligonucleotide (trinucleotide VIII) with larger acetylated oligonucleoside 5'-phosphate (such as hexanucleotide IX). After the usual work-up, the unreacted hexanucleoside 5'-phosphate can be easily removed by passing through a benzoylated DEAE-Cellulose column, whereas, the desired product, 2-phenylmercaptoethyl ester of 5'-phosphate nonanucleotide (X) and trinucleotide (VIII) are retained. These are then washed from the column with strong buffer containing 50% ethyl alcohol followed by successive purification on gel-filtration G-75 superfine and preparative tlc. The main advantage of this type of synthetic approach is that it allows an easier separation method of the final product from the starting material as the chain length of the oligonucleotide increases.

The enzymatic joining of (I) and (II) in the presence of a appropriate strand of T4-DNA is under investigation.

References

1. E. Terzaghi, Y. Okada, G. Streisinger, J. Emrich, M. Inouye and A. Tsugita, Proc. Natl. Acad. Sci., 56, 500 (1966).
2. M. Nirenberg, P. Leder, M. Bernfield, R. Brimacombe, J. Trupin, F. Rottman and C. O'Neal, Proc. Natl. Acad. Sci., 53, 1161 (1965).
3. H.G. Khorana, H. Buchi, H. Ghosh, N. Gupta, T.M. Jacob, H. Kossel, R. Morgan, S.A. Narang, E. Ohtsuka and R.D. Wells, Cold Spring Harbor Symp. Quant. Biol. 31, 39 (1966).
4. Y. Okada, G. Streisinger, J. (Emrich) Owen, J. Newton,

A. Tsugita and M. Inouye, Nature, 236, 338 (1972).
5. Thomas H. Jukes, In "Molecules and Evolution", p. 145, Columbia University Press, New York, 1966.
6. T. Kasai, E.K.F. Bautz, A. Guha and W. Szybalski, J. Mol. Biol. 34, 709 (1968).
7. C.R. Thomas, Jr. in "Progress in Nucleic Acid Research and Molecular Biology" (J.N. Davidson and W.E. Cohen ed.) vol. 5, p. 328, Academic Press, New York, 1966.
8. A.H. Ford-Moore, R.A. Peters and R.W. Wakelin, J. Chem. Soc. 1754 (1949).
9. J.J. Michniewicz, O.S. Bhanot, J. Goodchild, S.K. Dheer, R.H. Wightman and S.A. Narang, Biochim. Biophys. Acta, 224, 626 (1970).
10. S.A. Narang, O.S. Bhanot, J. Goodchild, R.H. Wightman, and S.K. Dheer, J. Amer. Chem. Soc., in press (1972).
11. S.A. Narang and J.J. Michniewicz, Anal. Biochem., in press (1972).
12. I. Gillam, S. Milward, D. Blew, M. von Tigerstrom, E. Wimmer and G.M. Tener, Biochem. 6, 3042 (1967); S.A. Narang, O.S. Bhanot, J. Goodchild and R.H. Wightman, Chem. Comm. 91 (1970); S.A. Narang, O.S. Bhanot, J. Goodchild, J.J. Michniewicz, R.H. Wightman and S.K. Dheer, Chem. Comm. 516 (1970).
13. S.A. Narang, J.J. Michniewicz and S.K. Dheer, J. Amer. Chem. Soc. 91, 936 (1969).
14. S.A. Narang, O.S. Bhanot, S.K. Dheer, J. Goodchild and J.J. Michniewicz, Biochem. Biophys. Res. Comm. 41, 1248 (1970).
15. R.H. Wightman, S.A. Narang and K. Itakura, Cand. J. Chem., 50, 456 (1972).
16. S.A. Narang, K. Itakura and R.H. Wightman, Cand. J. Chem., 50, 769 (1972).