BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 49(1), 349—350 (1976)

Template-Directed Synthesis of Oligonucleotide. Condensation of Nucleotide in the Presence of Polystyrene-Supported Neutral Oligonucleotide Derivative

Takeo SHIMIDZU and Akio IMAI

Department of Hydrocarbon Chemistry, Faculty of Engineering Kyoto University, Kyoto 606

(Received August 7, 1975)

Synopsis. A template-directed synthesis of oligonucleotide in the presence of polystyrene-supported neutral oligonucleotide derivative was studied. The oligonucleotide which has the sequence of the complementary base with the template, the neutral oligonucleotide derivative, yielded with a considerable amount, when the template with degree of oligomerization of 4 was used.

Chemically, we have two ways to synthesize the definite-sequence oligonucleotide: One is the stepwise synthesis of the oligonucleotide and the other is the template-directed synthesis. Several studies relating to the latter, the oligonucleotide syntheses using polynucleotides have been done,[1-8] but they were not necessarily successful.

Observations of those polynucleotide template-directed syntheses indicate a few problem. The complex formation between polynucleotide and condensing nucleotide in aqueous solution is not strong. Generally, that the equilibrium constant of the complex formation between nucleotide bases is remarkable in the aprotic solvent can be seen in IR[9] and NMR[10] studies. In addition, negatively charged phosphate groups both in the polynucleotide and the condensing nucleotide are undesirable for the complex formation. Disadvantage in the chemical reaction of the charged molecules on the polyelectrolyte having the same signed charge has been suggested by Ise.[11]

On the basis of those informations, a novel template-directed synthesis of the oligonucleotide has been tried.

The material was synthesized by the method of so-called solid support.[12] In the present study, the cross-linked polystyrene anchored oligonucleotide of which phosphoric acid moiety was esterified by cyanoethyl alcohol was used. This material was synthesized by the method of the preceding paper.[13] The oligonucleotide derivative in the present template was neutral at its phosphate moiety and anchored in the polystyrene matrix. Figure 1 shows an example of the template. The anchored oligonucleotide derivative has a failure in its sequence, in part.

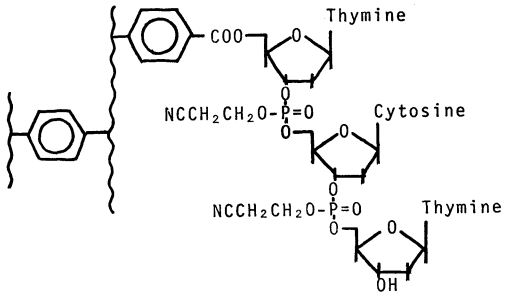

Fig. 1. [PSt]-TCT

Table 1 lists the result of the template-directed condensation of the nucleotide. In this condensation system, it is remarkable that the complementary oligonucleotide whose degree of condensation was more than two was obtained. However, the fully complementary oligonucleotide was scarecely obtained.

TABLE 1. CONDENSATION OF NUCLEOTIDE IN THE PRESENCE OF POLYSTYRENE-SUPPORTED NEUTRAL OLIGONUCLEOTIDE DERIVATIVE

Template	Nucleotide (mol%)	Relative yield of main product (optical density)
[PSt]-CH_3	pT(50) pdA(50)	pTpT(32) pdApdA(53) pTpdA(33) pdApT(38)
[PSt]-TTT[a]	pdA(100)	pdApdA(132) TpTpTpdA(66)
[PSt]-TTT[a]	pT(25) pdA(25) pdC(25) pdG(25)	pTpT(3) pdApdA(41) pdCpdC(34) pdGpdG(43) pTpdA(36) The others(62)
[PSt]-TTT[a]	pT(100)	pTpT(82)
[PSt]-TCT[b]	pT(25) pdA(25) pdC(25) pdG(25)	pTpT(11) pdApdA(41) pdCpdC(32) pdGpdG(65) pTpdA(32) The others(71) pdApdGpdA(0)
[PSt]-TCTC[c]	pT(25) pdA(25) pdC(25) pdG(25)	pTpT(14) pdApdA(49) pdCpdC(42) pdGpdG(84) pTpdA (29) pdApdGpdA (22) The others (88) Tetramer?(0)
[PSt]-TCTC[c]	pdA(50) pdG(50)	pdApdA (78) pdGpdG(132) pdApdG (175) pdGpdA(180) pdApdGpdA(39) pdGpdApdG(15) Tetramer ? (7)

a) TTT=96 mg/0.5 g-polymer support, % of the full sequence=79. b) TCT=85 mg/0.5 g-polymer support, % of the full sequence=78. c) TCTC=78 mg/0.5 g-polymer support, % of the full sequence=61. Total nucleotide (pyridinium form)=100 mg (calcd. as free acid). Pyridine=15 ml. Total DCCD=200 mg. Reaction time=24 hr. Room temperature.

In cases of [PSt]-CH_3 and [PSt]-TTT used, the rate of condensation of pdA was relatively high. In the product, the oligonucleotide which was additional product of the template oligonucleotide molecule, TpTpTpdA for example, was found. When four kinds of condensing nucleotides, pT, pdA, pdC, and pdG, were condensed in the presence of [PSt]-TTT or [PSt]-TCT, the maximum yield in the product was pdGpdG. And, in the case of [PSt]-TCT used, the fully complementary product, pdApdGpdA, was not found. On the other hand, when the tetramer template such as [PSt]-TCTC was used, though the fully complementary oligonucleotide, pdApdGpdApdG, was not found in the product, the partly complementary oligonucleotide yielded with a considerable amount. The yield of the partly complementary oligonucleotide appeared to be more prominent in the case that two kinds of condensing nucleotides, pdA and pdG, were used. In addition, the result showed that the yield of the oligonucleotide which was partly complementary with the template sequence close to the polystyrene solid support, *i.e.*, pdApdGpdA, was higher than that which not close to, *i.e.* pdGpdA-pdG. This shows the importance of a matrix constructed by the polymer that gives a hydrophobic enviroment.

Those results could lead the template-directed synthesis of oligonucleotide.

Support in part for this work by the Grant from the Ministry of Education (Grant in aid for Special Project Research 820828 and 911013). Thanks are due to Profeesor Kenichi Fukui for his encouragement. The authors are grateful to Dr. H. Hirai, Nagase Ind. Co., for kindly renting the Peptide Synthetizer Instrument (Schwartz Bioresearch).

References

1) R. Naylor and P. T. Gilham, *Biochemistry*, **5**, 2722 (1966).

2) J. Sulston, R. Lohrmann, L. E. Orgel, and H. T. Miles, *Proc. Nat. Acad. Sci., U. S.*, **60**, 409 (1968).

3) T. Shimidzu, and K. Fukui, *Annual Report Res. Inst. Chem. Fiber, Japan*, **25**, 87 (1968).

4) J. Sulston, R. Lohrmann, L. E. Orgel, H. Schneider-Bernloehr, B. J. Weimann, and H. T. Miles, *J. Mol. Biol.*, **40**, 227 (1969).

5) H. Schneider-Bernloehr, R. Lohrmann, J. Sulston, L. E. Orgel, and H. T. Miles, *J. Mol. Biol.*, **47**, 257 (1970).

6) Z. A. Shabarova, and M. A. Prokofiev, *FEBS Lett.*, **11**, 237 (1970).

7) S. Uesugi and P. O. P. Ts'O, *Biochemistry*, **13**, 3142 (1974).

8) L. E. Orgel, and Lohrmann, *Accounts Chem. Res.*, **7**, 368 (1974).

9) Y. Kyogoku, R. C. Lord, and A. Rich, *Science*, **154**, 518 (1966); *J. Amer. Chem. Soc.*, **89**, 496 (1967); *Proc. Nat. Acad. Sci. U. S.*, **57**, 250 (1969).

10) U. Krüger, H. Breyer, F. M. A. Klein, H. H. Perkampus, and K. H. Scheit, *Z. Naturforsch.*, **23B**, 1360 (1968).

11) N. Ise, *Nature*, **225**, 66 (1970).

12) R. L. Letsinger, and V. Mahadevan, *J. Amer. Chem. Soc.*, **87**, 3526 (1966); F. Cramer, R. Helbig, H. Hettler, K. H. Scheit, and H. Seliger, *Angew. Chem., Int. Ed.*, **5**, 601 (1966); G. M. Blackburn, M. J. Brown, and M. R. Harris, *J. Chem., Soc. C*, **1967**, 2438; L. R. Melby, and D. R. Strobach, *J. Amer. Chem. Soc.*, **89**, 450 (1969).

13) T. Shimidzu, and R. L. Letsinger, *J. Org. Chem.*, **33**, 708 (1968); This Bulletin, **44**, 1673 (1971).