DNA-like duplexes with repetitions: efficient template-guided polycondensation of decadeoxyribonucleotide imidazolide

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Received 21 February 1983

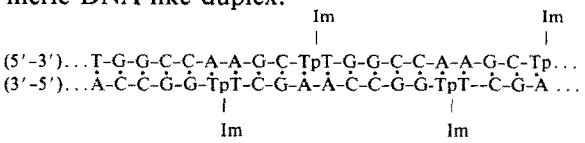
Abstract and keywords not received

1. INTRODUCTION

The only known way of constructing synthetic genes is the enzymatic ligation of the initial oligodeoxyribonucleotides in complementary duplex. Quite a logical approach to the total chemical synthesis of long-chain double-stranded polynucleotides is to condense the preformed oligomers by means of chemical condensing agents. Several groups have tried to create this technique for synthetic purposes [1–3], to investigate its relevance to prebiotic polynucleotide synthesis [4] or to mimic enzymatic reactions catalysed by polynucleotide ligases [5]. Unfortunately all attempts using the strategy of complementary block condensation were not promising for synthetic purposes.

Our laboratory has extensively investigated the preparation of concatemeric DNA-like duplexes [6]. We have developed a template-directed chemical condensation system for oligodeoxyribonucleotides with 3′-terminal phosphates [6,7]. The reaction was conducted at low temperatures in aqueous solution with water-soluble carbodiimide as the activating agent. The overall yield of 20–100-mer polynucleotides exceeded 90% [7].

The main goal of this research was to elaborate an effective procedure for chemical condensation of oligonucleotides with preactivated terminal 3′-phosphate group. The imidazole moiety was chosen for activation of phosphate group because phosphorimidazolides are known to be good phosphorylating agents in chemical [4,8] and enzymatic reactions [9,10]. The study of template-guided condensation was made with pseudopolymeric DNA-like duplex:



formed as a result of self-association of synthetic d(TGGCCAAGCTp-Im). Here, we report the results of the effective polycondensation of decanucleotide-3′-phosphorimidazolide into long-chain double-stranded polynucleotides.

Abbreviations: Im, imidazole moiety; MeIm, methylimidazole; Py, pyridine; DMAP, *N,N*-dimethylaminopyridine; Mcc, microcolumn chromatography; VPDE, snake venom phosphodiesterase; BAP, *E. coli* alkaline phosphatase.

2 MATERIALS AND METHODS

Decanucleotide d(TGGCCAAGCTp) (I) was synthesised in solution by the triester method as in [11]; imidazolide of decanucleotide d(TGGCCAAGCTp-Im) (II) was prepared by the carbodiimide method as in [12] with the yield >90–95%; VPDE and BAP were from Worthington Biochemical Corp.; all other reagents were from Merck (FRG).

The reaction mixture containing 0.3–0.4 A_{260} of (II) dissolved in 30 μl buffer (composition in table 1) was allowed to stand under the conditions in table 1. After 1, 4 and 12 days samples were withdrawn and subjected to Mcc-analysis on Lichrosorb-HN_2 resin.

Published by Elsevier Science Publishers
00145793/83/0000–0000/$3.00

Table 1

Condensation of d(TGGCCAAGCTp-Im)

Reaction mixture no.	Components of buffer (M)					pH	T (°C)	Condensation products (% yield)		
	Na-phosphate	NaCl	$MgCl_2$	HIm	MeIm			1 day	4 days	12 days
1	0.05	0.2	0.75	–	–	7.4	6	2–3	2–3	2–3
2	0.05	0.2	0.75	–	–	5.7	0	0	0	0
3[a]	0.05	0.2	0.75	–	–	7.5	6	10	12	15
4	–	0.2	0.12	0.2	–	8.0	6	0	0	0
5	–	0.2	0.12	–	0.05	8.0	6	15	30	50
6	–	0.2	0.12	–	0.1	8.0	6	47	53	61
7	–	0.1	0.06	–	0.1	7.4	6	33	42	–
8	–	0.1	0.06	–	0.1	8.0	6	46	78	91
9	–	0.2	0.12	–	0.2	8.0	6	67	73	85
10	–	0.2	0.12	–	0.2	8.0	10	61	80	84
11	–	0.2	0.12	–	0.2	8.0	20	35	40	50
12	–	0.2	0.12	–	0.2	9.0	6	40	44	54
13[b]	–	0.2	0.12	–	0.4	8.0	6	0	0	0
14	–	0.2	0.12	–	0.4	8.0	6	73	75	87
15	–	0.2	0.12	–	0.4	8.0	10	71	72	83
16[c]	–	0.2	0.12	–	–	9.0	6	2–3	5	12

Reaction mixture contained additional components: [a] 0.001 M Py; [b] 30% (v/v) formamide; [c] 0.01 M DMAP

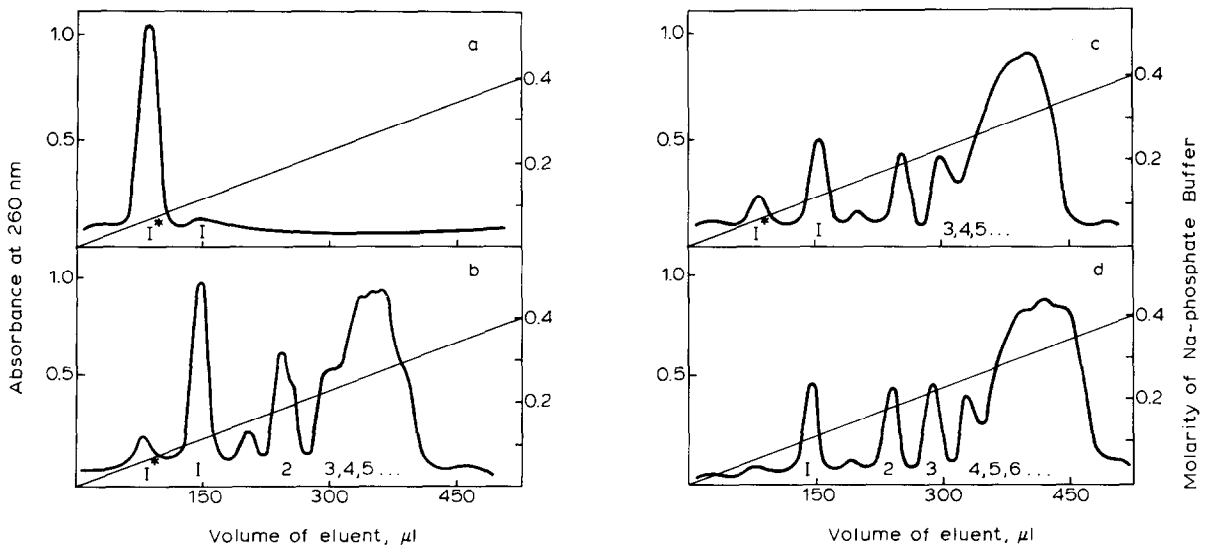


Fig. 1. Chromatography on Lichrosorb-NH_2 of reaction mixture no. 9 (table 1): before condensation (a); after 1 day (b); 4 days (c); 12 days (d) of incubation; 1 × 40 mm column, Na-phosphate gradient (pH 7.0) in 7 M urea, 20°C. Elution rate, 300 µl/h. Peak 1*, d(TGGCCAAGCTp-Im); peaks 1,2 ..., $d(TGGCCAAGCTp)_n$ where n corresponds to the number of the peak.

Yields of polycondensation products were determined as a ratio of the polycondensation products peak area to the total nucleotide peak area. The products were isolated by chromatography on Lichrosorb-HN_2 followed by gel-filtration on BioGel P-2. Digestion with VPDE and BAP was done as in [7], gel-electrophoresis as in [6,11]. The samples for electrophoresis contained 0.2–0.3 A_{260} of the nucleotide material. The gel slabs were stained with 0.05% 'Stains-all' in 50% formamide. The temperature dependence of UV absorbance was recorded with a Cary-219 spectrophotometer in thermostatted quartz cuvettes (Helma, FRG) with 1 mm pathlength as in [8]. The nucleotide concentration/monomer was 10^{-3} M.

3 RESULTS AND DISCUSSION

According to the optical data the conditions (table 1) chosen for polycondensation of (II) permit the formation of stable complementary duplex with T_m 25–27°C and hypochromicity $h = 20\%$. Thus the covalent binding of the imidazolide moiety to oligonucleotide does not cause the destabilisation of duplex.

The reaction mixtures containing (II) were incubated in the conditions of table 1; the composition of the buffers, pH and temperature were varied. Analysis of the reaction mixtures showed that polycondensation of (II) into di-, tri- etc. -meric blocks is efficient only in buffers containing MeIm, the yield of products exceeding 80% (table 1: 8–10; 14; 15; fig. 1). Polycondensation of (II) in the presence of other tertiary amines is less efficient (table 1: 3,4,16); in the absence of the tertiary amines no reaction occurs (table 1: 1,2). Further investigation of the role of different tertiary amines in the polycondensation of (II) is in progress. The optimal pH of the reaction was found to be 8.0, the decrease of pH to 5.7–6.0 enhances the hydrolysis of imidazolide (II) to decanucleotide (I) (table 1: 1,2,7–9,12).

The stability of the duplex is an indispensable condition of effective polycondensation. It was proved by the decrease of yields of polymeric products at an increase from 6–20°C (table 1: 9–11,14,15). Under the conditions causing total denaturation of duplex (table 1: 13), only hydrolysis of d(TGGCCAAGCTp-Im) to d(TGGCCAAGCTp) occurs.

The condensation products were analyzed by gel-electrophoresis and enzymatic digestion. The slag-gel electrophoresis of total polymeric fraction from table 1: 8–12,14,15 showed that it consists of d(TGGCCAAGCTp)$_n$, $n = 2$–7 (fig. 2a). The polymers were identical to the products of carbodiimide-induced condensation of d(TGGCCAAGCTp) (fig. 2b–d) obtained as in [8]. Decanucleotide (I) formed as a result of hydrolysis of the phosphoimidazolide bond is a terminator for a growing polynucleotide chain. The degree of

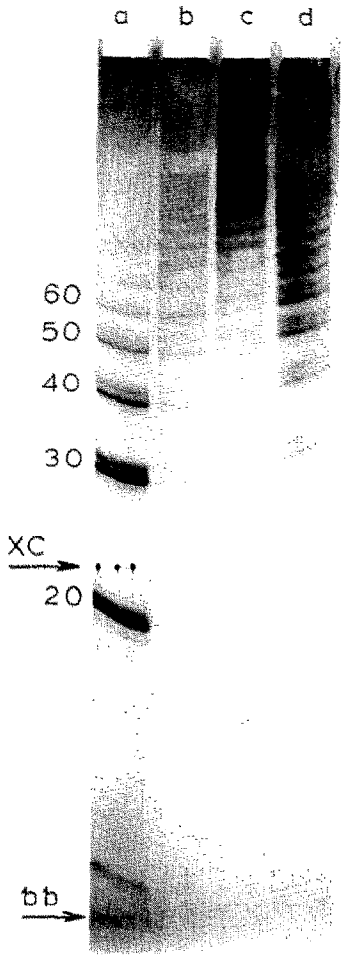
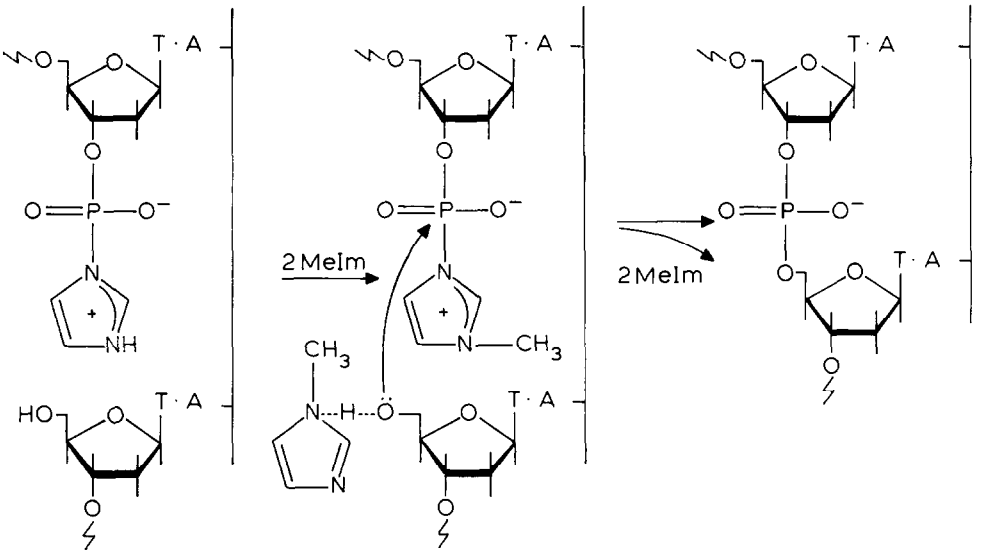


Fig. 2. Electrophoresis in 20% polyacrylamide gel of reaction mixtures 5–12,14,15 (table 1) (track a); products of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide-induced condensation of d(TGGCCAAGCTp), (tracks b–d). The numbers on the left indicate monomeric units in the oligonucleotide, XC, bb, position of marker dyes xylencyanol and bromphenol blue, respectively.

polymerization may be increased up to 10–12 by synthesizing imidazolide derivatives of d(TGGCC-AAGCTp)$_n$ (n = 2–7) obtained previously and submitting them to further condensation under experimental conditions (table 1: no. 10). The degradation of the products of polycondensation (table 1: 5–12,14,16) with VPDE and BAP gave only a mixture of nucleosides, thus confirming that the material formed is (3′–5′)-linked.

The mechanism of the formation of interoligomeric bond is not yet known, but we should like to emphasize that the rate of condensation of (II) is greatly enhanced by MeIm (table 1: 5,6,9,14) and is not sufficiently influenced by other tertiary amines. These results as well as some experimental data obtained in our laboratory enable us to suggest that MeIm functions as a nucleophylic [13] and at the same time general basic catalysts. On one hand MeIm transaminates the protonated form of phophorimidazolide (II), on the other it acts as an acceptor of proton from the neighbouring 5′-OH group:



This mechanism can operate only under the conditions that permit the formation of stable duplex and therefore provide a steric neighbourhood of reacting groups. In the case of concatameric duplex studied here the mechanism works rather well.

We have thus achieved an effective polycondensation of synthetic decanucleotide by means of imidazolide activation of its 3′-terminal phosphate. This approach of chemical condensation of oligonucleotides may have several advantages over the enzymatic ligation:

(i) It may be applied to a large scale synthesis of DNA-duplexes in amounts sufficient for instrumental study (e.g., (X-ray, NMR) of DNA;
(ii) The chemical method may be used in synthesis of double-stranded polynucleotides with unnatural sugar-phosphate backbones as well as modified heterocyclic bases.

ACKNOWLEDGEMENTS

We thank Dr L.E. Orgel (The Salk Institute for Biological Studies, San-Diego CA) for attention and information which was of great value to us.

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