Volume 232, number 1, 153–155 FEB 05843 May 1988

Chemical reactions within DNA duplexes

Cyanogen bromide as an effective oligodeoxyribonucleotide coupling agent

N.I. Sokolova, D.T. Ashirbekova, N.G. Dolinnaya and Z.A. Shabarova

A.N. Belozersky Laboratory and Chemistry Department, Moscow State University, Moscow 119899, USSR

Received 5 March 1988

Cyanogen bromide was found to condense oligodeoxyribonucleotides on a complementary template in aqueous solution. Optimum conditions for this vigorous and effective reaction were developed. CNBr proved to be useful for incorporation of phosphoramidate or pyrophosphate internucleotide bonds in DNA duplexes.

Cyanogen bromide; Chemical ligation; Modified DNA duplex; Internucleotide bond

1. INTRODUCTION

Template-directed condensation of oligodeoxyribonucleotides with the use of chemical coupling agents (chemical ligation) is one of the possible procedures for obtaining extended DNA duplexes [1]. The significance of this method has increased greatly as a result of its application to modified DNA synthesis [2,3]. In contrast, T_4 DNA ligase was shown to catalyze the assembly of natural DNA duplexes exclusively [2].

The most convenient and handy technique of chemical ligation is the use of condensing agents for activation of the phosphate groups in a nick. Until now, water-soluble carbodiimides have been almost exclusively used for this purpose, although their application is restricted by the slow reaction rate (0.2–6 days) and by the significant danger of base modification in single-stranded nucleic acids.

Here, we report on the use of CNBr as a coupling agent for the sealing of single-strand breaks in DNA duplexes. Although this reagent has been successfully applied to activate polysaccharide sorbents, it is not yet widely used in nucleotide chemistry. Thus far chemical reactions of CNBr with mononucleotides [4] as well as application for the modelling of prebiotic synthesis of ribonucleotide acids [5] have been described.

Correspondence address: Z.A. Shabarova, A.N. Belozersky Laboratory, Moscow State University, Moscow 119899, USSR

2. MATERIALS AND METHODS

Oligodeoxyribonucleotides were synthesized by the phosphoramidate method on a Victory 4M synthesizer, as in [6]. ACGGATp was obtained from ACGGArU by periodate oxidation of the 2′,3′-*cis*-hydroxyl system followed by β-elimination [7]. ACGGAT$_{NH_2}$ was obtained from ACGGAT$_{N_3}$, as described [8]. CNBr from Merck was distilled before use.

2.1. *Template-directed condensation of oligodeoxyribonucleotides using CNBr*

(i) An equimolar mixture of hexa-, undeca- and template tetradecanucleotides forming duplexes Ia–d in 9 μl buffer I (0.25 M 2-morpholinoethanesulfonate adjusted with Et_3N to pH 8.0, containing 0.02 M $MgCl_2$) was cooled to 0°C and then 1 μl of 2 M CNBr solution in absolute dimethylformamide was added. In the reaction mixture the oligomer concentration (per monomer), C_0, was 0.01 or 0.1 mM, with CNBr at 1000-fold excess over C_0. After 1 min the oligonucleotide fraction was precipitated with ethanol, dried and analyzed by 20% polyacrylamide gel electrophoresis under denaturing conditions. 5′-^{32}P-labeled hexa- or undecanucleotide was added previously to the reaction mixture (~10–15 nCi ^{32}P).

(ii) d(TGGCCAAGCTp) was polymerized under the same conditions as for duplexes Ia–d.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

3. RESULTS AND DISCUSSION

In order to study CNBr-induced condensation of oligodeoxyribonucleotides on a complementary template, the DNA duplexes containing a single nick were used (scheme 1):

(3′---5′) G-C-C-T-A-G-G-T-C-C-T-C-A-C
(5′---3′) A-C-G-G-A-X Y-C-A-G-G-A-G-T-G-A-C
↑
I

a: X = Tp b: X = T c: X = 3′-NH_2-T d: X = Tp

Y = C Y = pC Y = pC Y = pC

NB: The symbol d is omitted; the arrow marks the site of the oligonucleotide junction

Scheme 1.

To optimize the conditions of the coupling reaction we varied the CNBr and oligonucleotide concentrations (from 10- to 10^4-fold excess of CNBr, C_0 10^{-4}–10^{-3} M), incubation time (0.5–60 min), and temperature (0–20°C). Maximum yield was attained at 10^3-fold excess of CNBr within 1–2 min at 0°C. Chemical ligation did not proceed in the absence of the template oligonucleotide, i.e. outside the duplex.

Fig.1 shows the results of the chemical ligation. It is obvious that the efficiency of nick ligation in duplex Ia (yield 96%) greatly exceeds that in duplex Ib (yield 35%). When treated with CNBr, duplexes Ic and Id gave rise to heptadecanucleotides containing phosphoramidate or pyrophosphate inter-oligonucleotide bonds (86 and 67% yields, respectively). The primary structure of heptanucleotides prepared by nick ligation in duplexes Ia and Ib was confirmed by Maxam-Gilbert analysis. The nature of the pyrophosphate and phosphoramidate bonds within ligated duplexes Ic and Id was established by selective cleavage upon treatment with trifluoroacetic anhydride and 15% acetic acid [2], respectively.

To prove that oligonucleotides are not modified by CNBr during the course of the coupling reaction, control experiments were carried out. First, an equimolar mixture of 5′-phosphorylated undeca- and hexanucleotides (components of duplex Ib) was treated with CNBr under conditions for chemical ligation and then desalted. The template tetradecanucleotide was subsequently

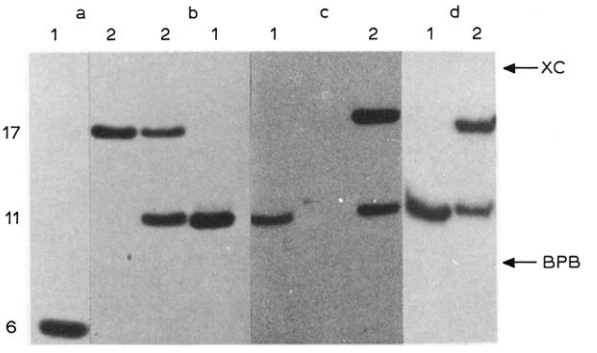

Fig.1. Autoradiogram of reaction mixture before (1) and after (2) CNBr-induced nick ligation in duplex Ia (a), Ib (b), Ic (c) and Id (d). For duplex composition see scheme 1. Conditions for coupling reaction given in section 2. The chain length of oligonucleotides is indicated. Markers: XC, xylene cyanol; BPB, bromphenol blue.

added to the mixture and the resulting nick-containing duplex was treated with T_4 DNA ligase. The efficiency of enzymatic ligation was about 100%. Second, the ligated duplex Ia, containing the *Eco*RII restriction endonuclease recognition site, was cleaved by this enzyme. The cleavage efficiency was the same as that for an analogous duplex obtained by enzymatic ligation (fig.2). It is essential that the newly formed bond was cleaved.

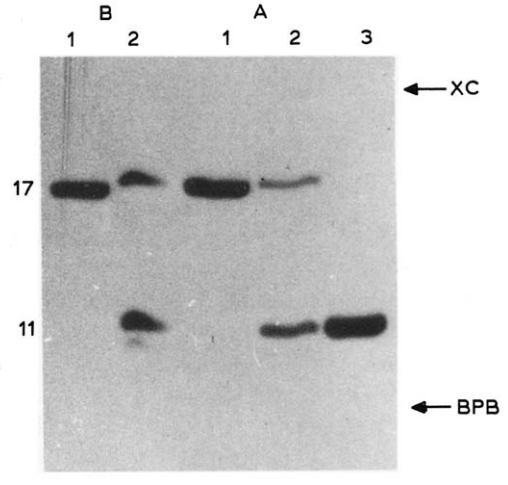

Fig.2. Autoradiogram of cleavage products of ligated duplex Ia with endonuclease *Eco*RII under the conditions in [9]. Initial substrates (1), reaction products (2), marker 5′-^{32}P-labelled d(pTCAGGAGTGAC) (3). Nick ligation was performed using CNBr (A) or T_4 DNA ligase (B). Conditions for enzymatic ligation: see [2].

Hence, if modification of the nucleotide residues or the interacting hydroxy- and phosphate groups did take place, it must be reversible and not result in the significant formation of by-products. The lack of modification products was also confirmed by the fact that no extra bands were detected on autoradiograms (fig.1).

It is interesting to use CNBr for assembling more extended DNA duplexes. Earlier it was shown that the self-association of d(TGGCCAAGCTp) in aqueous solution produces rather long DNA-like duplexes (concatemers) [9]. CNBr treatment of these duplexes for 1 min at 0°C induced polycondensation of decanucleotide (yield 85%). The length of the polynucleotides thus formed was a multiple of the length of the initial oligomer: $d(TGGCCAAGCTp)_n$ where $n = 2–16$ (not shown).

Thus, we have demonstrated a new application of CNBr as a condensing agent for chemical ligation. The significant advantages offered by CNBr as compared with the water-soluble carbodiimide normally used are that it is highly reactive and yields no modified products.

REFERENCES

[1] Shabarova, Z.A. (1984) in: Physicochemical Biology Reviews, Soviet Scientific Reviews, Section D (Skulachev, V.P. ed.) pp.1–51, Harwood.
[2] Dolinnaya, N.G., Gryaznova, O.I., Sokolova, N.I. and Shabarova, Z.A. (1986) Bioorg. Khim. 12, 755–763.
[3] Dolinnaya, N.G., Gryaznova, O.I., Sokolova, N.I. and Shabarova, Z.A. (1986) Bioorg. Khim. 12, 921–928.
[4] Ferris, J.P. and Yanagava, H. (1984) J. Org. Chem. 49, 2121–2125.
[5] Kanaya, E. and Yanagawa, H. (1986) Biochemistry 25, 7423–7430.
[6] Gryaznov, S.M., Chernov, I.P., Potapov, V.K., Purmal, A.A., Metelev, V.G., Yolov, A.A. and Shabarova, Z.A. (1986) Bioorg. Khim. 12, 1604–1611.
[7] Krynetskaya, N.F., Zayakina, G.V., Oretskaya, T.S., Volkov, E.M. and Shabarova, Z.A. (1986) Nucleosides Nucleotides 5, 33–43.
[8] Gryaznova, O.I., Dolinnaya, N.G., Isagulyants, M.G., Metelev, V.G., Oretskaya, T.S., Vdalov, N.I., Sokolova, N.I. and Shabarova, Z.A. (1986) Bioorg. Khim. 12, 124–132.
[9] Vinogradova, M.N., Gromova, E.S., Gryaznova, O.I., Isagulyants, M.G., Kuznetsova, S.A., Kosych, V.G. and Shabarova, Z.A. (1987) Bioorg. Khim. 13, 1194–1203.
[10] Shabarova, Z.A., Dolinnaya, N.G., Drutsa, V.L., Melnicova, N.P. and Purmal, A.A. (1981) Nucleic Acids Res. 9, 5747–5760.