The use of BrCN for assembling modified DNA duplexes and DNA-RNA hybrids; comparison with water-soluble carbodiimide

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ABSTRACT

Both cyanogen bromide (BrCN) and 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide may be used as coupling reagents for the template-directed assembly of DNA duplexes containing the sugar-phosphate backbone modification. Both reagents show similar ligation site structure-specific trend. Practical recommendations are given for selection of the condensing reagent depending on the properties of the duplex. Based on ^{31}P NMR spectroscopy data, a scheme is suggested for BrCN activation of the nucleotide phosphomonoester group. Using both condensing reagents, we studied the condensation of oligonucleotides containing ribo-segments (from mononucleotide residue to full sequence) on the DNA template. Efficiency of the chemical ligation of RNA oligomers was shown to be much lower than that of DNA analogues. The coupling yield depends on the position of the RNA segment in the hybrid duplexes and on the position of the phosphate group in the nick.

INTRODUCTION

Extensive studies of chemical reactions in DNA duplexes have made it possible to develop a procedure (alternative to the enzymatic method) for the assembly of extended double-stranded DNAs from oligonucleotide blocks using a chemical reagent (chemical ligation) [1].

The significance of chemical ligation has grown especially after demonstration of its possibility to incorporate various modifications of the sugar-phosphate backbone at a particular site when DNA duplexes are being assembled. T4 DNA ligase was shown [2] to catalyze the formation of natural internucleotide bonds exclusively.

The most convenient and handy technique of chemical ligation is the use of condensing agents for activation of the phosphate group in the 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 [2,3].

In 1987 we reported on the use of BrCN as a coupling agent for chemical ligation [4]. The main advantage of the latter is a high reaction rate and the absence of by-products.

Almost at the same time E.Kanaya and H.Yanagawa [5] have been demonstrated that BrCN condensed oligoadenylates on a poly(uridilic acid) template. The reaction proceded for 20–25 hours at 25°C and only in the presence of imidazole and divalent metal ions. The authors proposed that the true condensing agents in this reaction are N-cyanoimidazole and N,N'-iminodiimidazole formed rapidly from BrCN and imidazole.

In our procedure BrCN was found to condense oligodeoxyribonucleotides during 1 min in an aqueous solution. Apparently, the mechanism of nucleotide activation by BrCN herein and that reported in the work [5] are different.

In the present communication we employ the ^{31}P NMR spectroscopy for elucidation of reaction pathways of BrCN with the deoxyribonucleotides under our conditions.

Additionally, a comparison of the condensing ability and 'substrate specificity' of two condensing agents: 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (CDI) and BrCN has been carried out. For this purpose we used two series of DNA duplexes having different natures and orientations of the reacting groups in the nick (Scheme 1). Finally, we have examined the potentiality of BrCN and CDI for the template-directed coupling of RNA fragments or of RNA with DNA. So far chemical ligation has been used for assembling DNA duplexes or to mimic prebiotic polynucleotide synthesis. However, the elaboration of an effective procedure for ligation of RNA fragments is necessary for developing the recombinant RNA technique. The search for chemical ways of solving this problem is necessary because of the absence of enzymes that join oligo(poly)ribonucleotides on a complementary template. It is just the possibility for holding the two reacting ends, which may be involved in the formation of the complex secondary structure, close to each other, that makes template-directed reactions attractive. The structure of RNA-DNA hybrid duplexes employed in our study is presented in Table 1.

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MATERIALS AND METHODS

General

Used in the work were CDI, BrCN, MES [6], Tris, HEPES, acrylamide, N,N'-methylenbisacrylamide (Merck), γ-^{32}P-ATP (Amersham), RNase T_2 (Sankay), T4 DNA ligase, RNase A (USSR).

The following buffer solutions were used: buffer 1 (for CDI-induced ligation)—50 mM MES, pH 6.0, 20 mM $MgCl_2$; buffer 2 (for BrCN-induced ligation)—0.25 M MES—$(C_2H_5)_3N$, pH 7.5, 0.02 M $MgCl_2$; buffer 3 (for RNase A)—0.1 M Tris-HCl, pH 7.5, 2 mM EDTA; buffer 4 (for RNase T_2)—0.04 M ammonium acetate, pH 4.5, 0.002 M EDTA.

^{31}P NMR spectra

NMR spectra were recorded on a Bruker HXS-270 spectrometer operating at 121.5 MHz equipped with a variable temperature unit. The chemical shifts are given in ppm relative to 85% phosphoric acid as an external standard.

Other methods

Paper chromatography was performed by the ascending technique on a Whatman 1 in a system of ethanol—1 M ammonium acetate (7:3 v/v), pH 7.5.

Paper electrophoresis was carried in 0.05 M TEAB at pH 7.5 and 35 V/cm. Colorimetrical control for BrCN concentration under chemical ligation conditions was made as in [7].

Preparation of oligonucleotides

Oligodeoxyribonucleotides were synthesized by the phosphoramidite method on a Cyclon (Biosearch) DNA synthesizer using the standard β-cyanoethyl phosphoramidite cycle. Synthesis of d(ACGGAT-NH_2) was performed as described earlier [2]. Synthesis of oligomers with changed configuration at C2'or C3' atoms was described in the previous publication [8].

Oligoribonucleotides were kindly provided by R.Reinchoff (Institute of Organic Synthesis of Latvian Academy of Science).

5'-^{32}P phosphorylation of oligonucleotides and electrophoresis in 20% denaturing polyacrylamide gel were effected by the standard procedure.

3'-phosphorylated oligomers were prepared from oligomers containing 3'-terminal uridine by periodate oxydation of the 2',3'-cis-hydroxyl system followed by β-elimination [9].

Chemical ligation of oligonucleotides

Condensation of oligodeoxyribonucleotides in the duplexes I-II using CDI was carried out as described in [2].

Condensation of oligodeoxyribonucleotides in the duplexes I-II using BrCN (General Method). The reaction was carried out in a 2 ml Eppendorf tube. A mixture of oligonucleotides in 9 μl of buffer 2 was cooled to 0—5°C and then 1 μl of a 2 M BrCN (freshly distilled) solution in absolute DMF was added. In the reaction mixture the nucleotide concentration (per monomer), C_o, was 0.1 mM, BrCN—100 mM. After 1 min the oligonucleotide fraction was precipitated with ethanol, dried and analyzed by gel electrophoresis. 5'-^{32}P-labelled oligomers were added previously to the reaction mixture (~10—15 nCi ^{32}P) in a 1.5—fold deficiency as compared to the unlabelled ones.

Enzymatic ligation was carried out as described earlier [2].

Reactions of dpA with BrCN

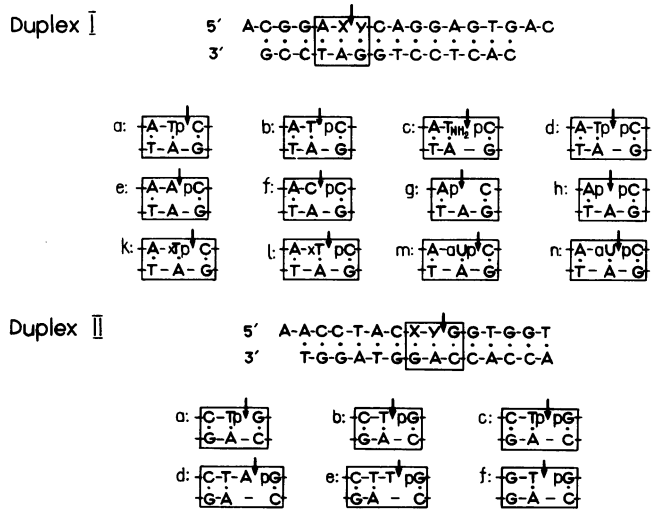
In aqueous solutions: to a solution of dilitium salt of dpA (77 mg, 0.225 mmol) in 850 μl buffer 2 at 0—5°C was added the solution of BrCN (120 mg, 1.125 mmol) in 150 μl abs. DMF and ^{31}P NMR spectra were recorded. Then reaction mixture was evaporated to remove the residual BrCN and was analyzed by paper chromatography and electrophoresis. R_f dpA=0.18, U_{dpA}=1.

In abs. DMF: to a solution of triethyl ammonium salt of dpA (65 mg, 0.15 mmol) in 750 μl abs DMF at 0—5° was added the solution of BrCN (80 mg, 0.75 mmol) in 100 μl abs DMF and ^{31}P NMR spectra were recorded. The reaction mixture was analyzed in the same way as described above. For deoxyadenosine pyrophosphate R_f = 0.25, U_{dpA} = 0.75.

RESULTS AND DISCUSSION

A ^{31}P NMR study of the reaction of BrCN with deoxyadenosine 5'-phosphate

Few experimental data on BrCN interaction with nucleosides and nucleotides are currently available. Only uridine derivatives were isolated and characterized [10]. The vicinal 2',3'-hydroxyl groups are essential for the reaction as shown by the failure to form stable reaction products with thymidine [10]. The phosphate derivatives of nucleotides have not been detected. However, our results show that formation of activated phosphate adduct probably occurs, because BrCN successfully joins 5'- and 3'-phosphorylated oligonucleotides in duplex Id (Scheme 1). To solve this problem, the ^{31}P NMR study of reaction between BrCN and dpA under different conditions was carried out. As the stability of BrCN in aqueous medium was low (T/2 < 1 min) we were forced to use an appreciable excess of this reagent. However, we have not succeeded in identifying a highly reactive intermediate. In the reaction mixture obtained by treatment of dpA in buffer 2 with 5 eqv. of BrCN the single signal was recorded in 1—2 min at 5°C, which we assigned to the initial dpA. This was confirmed by the paper chromatography data and electrophoresis analysis. The difference in the position of dpA resonances before



Scheme 1. The symbol d is omitted and the arrows indicate the single stranded break. Reactive site structure is given in the squares. For abbreviation dxT and aU see [6].

(δ_p=2.09 ppm) and after (δ_p= 1.05 ppm) the BrCN addition is caused by the pH value change of the reaction medium. The treatment of dpA (δ_p=1.8) with BrCN in abs. DMF resulted in conversion of dpA after 1−2 min to the compounds represented in the ^{31}P NMR spectrum by signals at -(1012) ppm and −22 ppm (Fig. 1). The signal at δ_p=−10.9 ppm may be assigned to adenosine pyrophosphate; two groups of signals at δ_p=−11.5 and −22 ppm are attributed to deoxyadenosine tripolyphosphate [11]. The chromatographic analysis confirmed the presence in the reaction mixture of deoxyadenosine pyrophosphate and dpA, which probably was formed due to the hydrolysis of the unstable deoxyadenosine tripolyphosphate.

It is more likely that BrCN does react with the phosphate group of dpA, the unstable intermediates being formed (in parentheses on Scheme 2). Water molecules hydrolyze the activated phosphate intermediate back to dpA. In abs. DMF it interacts with phosphate groups of other nucleotides to give the polyphosphates (Scheme 2). Apparently, only proximity of BrCN-activated phosphate and hydroxyl or other nucleophilic groups in the nick of the thermodynamically stable double helix provides successful formation of a new internucleotide bond.

Comparative study of BrCN and CDI as chemical ligation reagents

We showed earlier [2,8] that the efficiency of CDI-induced ligation strongly depends on the nucleophilicity of the reacting groups and on the conformation of the nicked site. To know, whether these dependences remain when BrCN is used, we carried out coupling reactions on duplexes I and II (Scheme 1) in a parallel series experiments using both condensing agents. Duplexes I and II differ in the nucleotide composition but are constructed according to the common principle: within each series the template and one of the oligomers complementary to it remain unchanged, whereas the second oligomer differs only at the nucleotide residue facing the nick. Modifications at the reaction site included replacement of the hydroxyl group by amino-(duplex

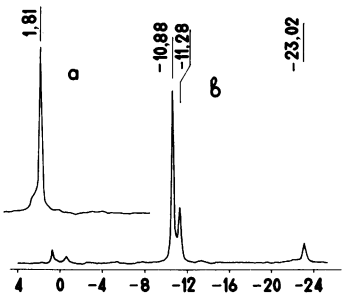
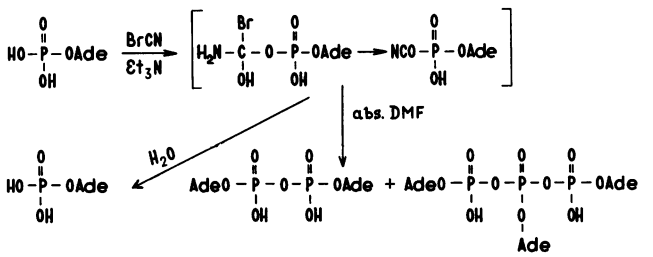


Figure 1. ^{31}P NMR spectra of dpA in abs. DMF (a) and of reaction products of dpA (0.15 M) with BrCN (0.75 M) in abs. DMF.



Ic) or phosphate groups (duplexes Id,h and IIc), of the terminal dT by dA or dC (duplexes Ie and If with dAdA or dAdC mismatches), of the dCdG base pair second to the single strand break with a noncomplementary dGdG pair (duplex IIf), omission of one nucleotide residue (duplexes Ig, Ih), incorporation of an extra base into the nick (duplexes IId,e) or replacement of the terminal dT by rU or nucleosides with configuration changes at C2′- or C3′ atoms (duplexes Ik-n).

The primary structure of oligonucleotides prepared by the nick ligation in duplexes I and II was confirmed by Maxam−Gilbert analysis, the nature of the resultant unnatural pyrophosphate (duplexes Id and Ih, IIc) or phosphoramidate (duplex Ic) bonds was proved by their selective cleavage, as described in [2]. The nature of the bond formed by rU (duplexes Io,p, Table 1), as well as of dxT (duplexes Ik,l) and aU (duplexes Im,n) was proved by the alkaline and enzymatic hydrolysis of the corresponding oligomers [8].

The yields of the products of the BrCN- and CDI-induced ligation are summarized in Table 2 and in Fig. 2. One can see that the internucleotide bond under the action of the both reagents is formed more readily if a reacting phosphate group is at the 3′-end of the oligomer (compare duplexes Ia and Ib, IIa and IIb in Table 2). Apparently, the activated 5′-phosphate has a greater

Table 1. Yields of chemical ligation products in hybrid RNA-DNA duplexes

Duplex number	Duplex composition*	Condensing agent** BrCN	CDI
Ib	5′ ACGGAT *pCCAGGAGTGAC 3′ GCCTA−GGTCCTCAC	35 (67)	75
Io	ACGGA(U) *pCCAGGAGTGAC GCCT A− GGTCCTCAC	16	35
Ip	*pACGGA(Up) CCAGGAGTGAC GCCT A−GGTCCTCAC	<5	<5
Ir	*pACGGA(Up) pCCAGGAGTGAC GCCT A - GGTCCTCAC	8	42
III	AATGG (*pAAAACCCAUG) TTTACC− TTTTGGGTACC	7	10
IV	*pAATGGp (AAAACCCAUG) TTTACC−TTTTGGGTACC	40 (74)	90
V	AATGGp (*pAAAACCCAUG) TTTACC - TTTTGGGTACC	35 (65)	85
VI	(AAUGG) (*pAAAACCCAUG) TTTACC− TTTTGGGTACC	4	16

* Symbol *d* is omitted, ribonucleotide units (blocks) are given in parentheses.
** Reaction time is 1 min for BrCN and 6 days for CDI; product yield after 3-fold addition of BrCN is indicated in parentheses.

Table 2. The results of chemical ligation of nicks in some DNA duplexes

Duplex number	Condensing reagent BrCN* Yield, %	CDI Yield, %	CDI Reaction time, days
Ia	95	95	4
Ib	35	75	6
Ic	86	97	0.25
Id	67	92	0.25
Ih	16	75	6
IIa	45	72	6
IIb	24	30	6
IIc	47	75	0.25

* Reaction time is 1 min everywhere.

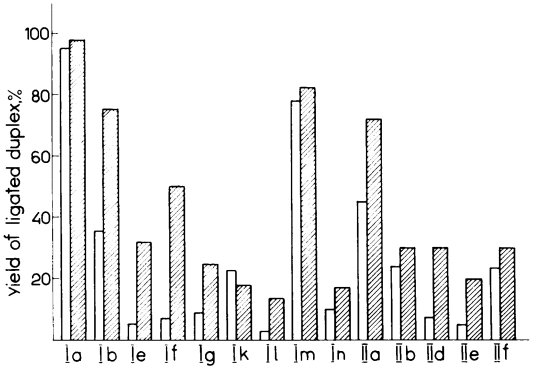


Figure 2. Plot of coupling yields *vs* nicked site structure (duplex numbers are indicated below. BrCN−induced ligation (open bars), CDI−induced ligation (shaded bars). For reaction conditions see Materials and Methods.

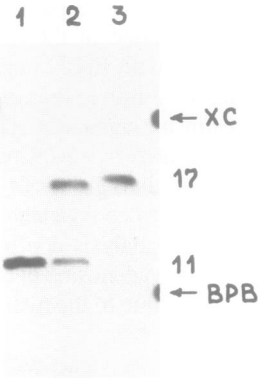


Figure 3. Polyacrylamide gel electrophoretic analysis of duplex Io before (1) and after (3) incubation with T4 DNA ligase; (2)−enzymatic nick ligation in duplex Io was carried out after pretreatment of its component d(ACGGA)rU with BrCN. The chain length of oligonucleotides is indicated to the right. The arrows indicate the positions of markers: xylene cyanol (XC) and bromophenol blue (BPB).

conformational flexibility and, consequently, exposure for hydrolysis. On the other hand, the activated 3′-phosphate is accepted by a much more reactive 5′-hydroxyl of the adjacent oligonucleotide molecule.

The substitution of a more nucleophilic amino or phosphate group for the hydroxyl group (duplexes Ic, Id and IIc) makes the CDI-induced ligation more effective, and yield of product approaches 70−90% not in 6 days as for duplex Ib, but in 6 hours. In case of BrCN as a condensing agent the nick ligation in the same duplexes is less effective (Table 2). It may be explained by the side reaction of BrCN with 3′-terminal NH_2-group of hexanucleotide (duplex Ic) or by blocking the nucleophilic attack because of BrCN interaction with both phosphate groups facing the nick (duplexes Id and IIc). In contrast, accumulation of the phosphate activated adduct does not occur if CDI is used as a condensing reagent [8].

The changes in the orientation and proximity of the reacting groups (their chemical nature remains preserved), caused by mismatched base pairs (duplexes Ie−Ih, IId−IIf) or by variations of sugar configuration (duplexes Ik−In), decrease the coupling yield (Fig. 2). The correlation of the action of both condensing reagents is rather obvious. This finding strongly supports the idea that the result of chemical ligation depends mainly on the reactive site structure. So, the reaction effectiveness indicates how much the conformation of the modified site differs from a canonical one. Apparently, the degree of exposure of BrCN- or CDI-activated phosphate groups to water molecules is essential here.

As can be seen in Fig. 2 the lowering of the coupling yield caused by the perturbation of the nick structure is much more considerable if the BrCN one is used. To improve the situation we have elaborated the following technique. After the end of the reaction (1 min), the nucleotide material was precipitated, dissolved in a new portion of the buffer, and fresh BrCN was added. By repeating such a procedure 2 or 3 times, we succeeded in appreciable intensification of the reaction even in 'unfavored' duplexes. Thus, after a single treatment of duplex Ib with BrCN the yield of ligation product was 35%, whereas after a three-fold treatment it became 68%.

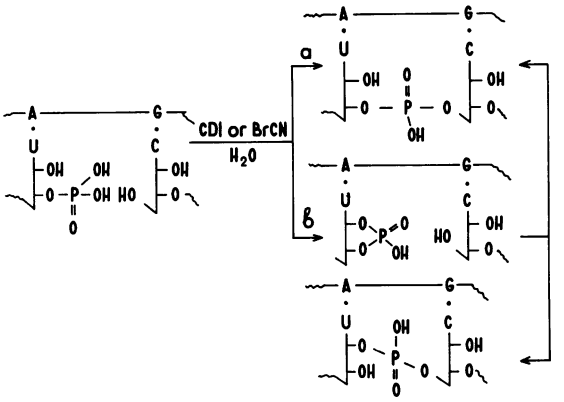
The data obtained indicate that each of the condensing reagents employed has its advantages and disadvantages. Thus, for the synthesis of unnatural internucleotide bonds such as pyrophosphate or phosphoramidate ones it is more expedient to use CDI. When assembling extended natural DNA, one should choose BrCN, because the rate of BrCN-induced coupling reaction is extremely high.

Assembling of RNA-DNA hybrid helix

It might be supposed that the template condensation of ribonucleoside units should proceed more effectively than the condensation of deoxyribonucleoside ones since the presence of a 2′-hydroxyl group in the RNA backbone makes it more reactive and less flexible than the DNA backbone. Nevertheless, as has been shown earlier [2], the yield of the products of the CDI-induced ligation lowers by a factor greater than two upon the replacement of thymidine by uridine in the coupling site. We have examined in detail the ligation of RNA fragments with DNA or RNA ones on a series of hybrid duplexes (Table 1). The oligonucleotides to be ligated differ in the number of the ribonucleotide units (from one to a complete ribonucleotide sequence). The resulting duplexes have the DNA-RNA hybrid in one (duplexes Io, Ip, Ir), ten (duplexes III−V) and fifteen (duplex VI) base pairs. The data obtained demonstrate (Table 1) that the effectiveness of coupling reaction induced by both CDI and BrCN is considerably lower in duplex Io containing uridine unit as a phosphate acceptor than in DNA-DNA duplex Ib. It may be due to conformational differences between ribo- and deoxyribonucleotide units in the double helix. It is known that sugar puckering mode for B-DNA is C2′-endo with a pseudo-axial orientation of the 3′-hydroxyl group. Meanwhile, in the hybrid duplex composed of covalently bonded RNA and DNA blocks the ribonucleotide units because of their structural conservatism display a sugar pucker close to the C3′-endo conformation [12]. In this case the pseudo-equatorial orientation of the 3′-hydroxyl group may sterically hinder its interaction with the phosphate group of the neighbouring molecule.

An additional reason for the lowering of the coupling yield in duplex Ia under the action of BrCN may be a modification of 2′- and 3′-hydroxyl groups of the 3′-terminal uridine, as described by Ferris at el. [10]. The possibility of this side reaction was confirmed by the following control experiment: the enzymatic ligation yield in duplex Io lowers from 90 to 60% if reacting hexanucleotide d(ACGGA)rU is pretreated with BrCN under the chemical ligation conditions (Fig.3).

Since the phosphate acceptor in duplex Io is uridine residue, the formation of both 3′-5′ and 2′-5′ internucleotide bonds may occur. To determine the isomer ratio, d(ACGGA)rUd(pCCAG-GAGTGAC) obtained by the BrCN-induced ligation was



hydrolyzed with RNase A (buffer 3, 1,5 h, 37°C). 86% of phosphodiester linkage between rU and dC was digested. Earlier the same percentage of the natural isomer was found in the product of the CDI-induced ligation in the same duplex [2]. In the control experiment the enzymatically obtained d(ACGGA)rUd(pCCAGGAGTGAC) was hydrolyzed with RNase A by 100%.

Duplexes Ip and Ir differ from duplex Io only by the number or position of the phosphate groups in the reaction site: in duplex Ip there is a 3′-phosphate group, in duplex Ir both ends of the broken chain are phosphorylated. The efficiency of chemical ligation in both duplexes is low (Table 1). Apparently, the activation of the 3′-phosphate group of the ribonucleotide unit leads to two competing reactions (Scheme 3): an intermolecular joining of oligomers (pathway 'a') and an intramolecular 2′,3′-cyclophosphate formation (pathway 'b').

The quantitative conversion of the 3′-phosphate group of d(ACGGA)rUp into 2′,3′-cyclophosphate after the BrCN treatment confirms that the reaction along the pathway 'b' takes place. Probably, it is this side reaction that prevents effective coupling in duplexes Ip and Ir. It should be noted that a new internucleotide bond in these duplexes may be formed not only along the pathway 'a' but also along the pathway 'b', since 2′,3′-cyclophosphates are rather active derivatives and they may react with the 5′-hydroxyl or 5′-phosphate group of the neighbouring oligomer, giving a mixture of 2′-5′- and 3′-5′-linked isomers [13]. The ratio of the reaction products will depend on the duplex stability and the conformation of the nicked site.

The situation is slightly improved if the second phosphate group is introduced into the nick (duplex Ir) (Table 1). However, the yield of the product with a pyrophosphate bond between the oligomers in this duplex is far from 100% ones easily attainable in the chemical ligation of DNA blocks with 3′- and 5′-phosphate groups in the reaction site [2].

Hybrid duplexes III–V, in which deoxyribo- and ribooligomers are joined on the DNA-template, have the same primary structure and differ only in the position or number of the phosphate groups in the coupling site. As should be expected, in these duplexes joining of 3′-phosphorylated DNA blocks with oligoribonucleotides carrying the 5′-hydroxyl (duplex IV) or 5′-phosphate groups (duplex V) proceeded most effectively (Table 1 and Fig.4). It is interesting, that T4 DNA ligase only poorly joins deoxyoligomers to ribooligomers to give the dN_irN_k product [14]. It seems reasonable that a disruption of the helix at the junction of two conformation: B-form for DNA-DNA duplex end and A-form for RNA-DNA hybrid end [14], slightly decreases the block condensation efficiency.

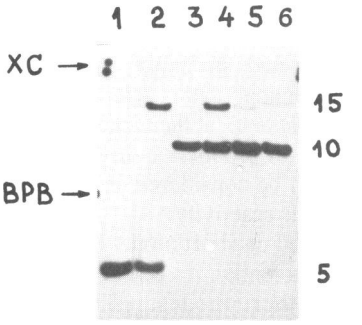


Figure 4. Polyacrylamide gel electrophoretic analysis of the reaction mixture after BrCN-induced nick ligation in duplexes IV (2), V (4), III (5) and VI (6). For conditions see Materials and Methods. Lanes (1) and (3) correspond to the oligomers d(*pAATGGp) and r(*pAAAACCCAUG). Designations as in Fig.1.

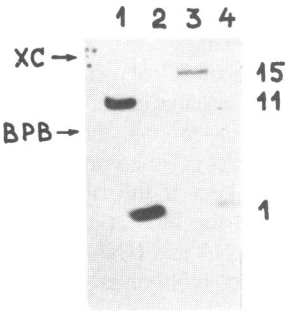


Figure 5. Polyacrylamide gel electrophoretic analysis (1) control $r(A_4C_3AUG*pCp)$, (3) $r(A_2UGG*pA_4C_3AUG)$, obtained by BrCN-induced ligation in duplex VI; (2), (4)–RNase T_2 hydrolysis products of these oligomers (buffer 4, 2 h, 37°C).

The template-directed joining of the RNA fragment with the use of both chemical reagents are sufficiently poor (duplex VI in Table 1). This result agrees with the data described above that ribonucleotide units are not efficient acceptors of the activated phosphate group. In the double helix they adopt C3′-endo conformation unfavorable for chemical reactions. Certainly, one cannot exclude also the influence of the secondary structure of the duplex on the effectiveness of the joining of RNA fragments. It has been calculated that accessibility of phosphate oxygen atoms to the solvent (and, hence, to the condensing reagent) in the A-type of the double helix to which the RNA-DNA hybrid belongs, is twice lower than in the B-type [15]. Furthermore, in the A-form the adjacent phosphate groups are closer together than they are in the B-DNA (5.9 and 7.0 Å respectively [15]). It may affect the steric factors of the chemical reactions.

To determine the type of the internucleotide bond synthesized upon the chemical ligation of oligoribonucleotides (duplex VI), RNase T_2 was used. As seen in Fig.5, the degradation of r(AAUGGpAAAACCCAUG) with this enzyme gave only a mixture of mononucleotides thus confirming that the material formed is (3′-5′)-linked. The natural phosphodiester bond is formed with the both condensing reagents.

CONCLUSION

The successful application of both reagents for obtaining DNA duplexes with various sugar phosphate backbone modifications, as well as DNA-RNA hybrids have been demonstrated.

It turned out that the efficiency of chemical ligation depends little on the nature of condensing reagents, even such different in their composition and, probably, in the reaction kinetics as BrCN and CDI. Coupling yield is determined mainly by the local structure of the double helix at the nicked site. Although the yield of BrCN condensation products is lower compared to that for CDI, the former can be considered as a promising reagent due to its extremely high reactivity.

The results obtained on short model RNA-DNA hybrids make one recognize that potentialities of chemical ligation of a RNA fragments are rather modest. The replacement of a deoxyribonucleotide unit by a ribonucleotide one in the nick leads to a sharp drop in the coupling yield. This effect is independent of whether the ribo-unit is a phosphate donor or acceptor. If the phosphate group is located at C3′ atom, oligomer joining is accompanied by a side reaction of the not quite active 2′,3′-cyclophosphate formation. But then the ribonucleotide unit behaves as a phosphate acceptor, lowering of the reaction efficiency is associated, probably, with the unfavorable N-type conformation of ribose ring.

Apparently, the ratio of (2′-5′)- and (3′-5′)-linked isomers strongly depends on the orientation of the reacting groups at the single-strand break. As far as the ligation of ribo- and deoxiribonucleotide blocks is concerned, all negative regularities pointed out above are kept if the ribonucleotide block is the 5′-terminal fragment of the block polymer. In the case of the opposite orientation of RNA and DNA blocks with the phosphate group localized on the deoxyoligomer, the efficiency of chemical ligation is high.

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6. Abbreviations: MES, 2-morpholinoethane sulfonate; T-NH_2, 3′-amino-3′-deoxythymidine; dxT, 1-(β-D-2′-deoxy-*threo*-pentofuranosyl) thymine; aU, 1-(β-D-arabinofuranosyl)uracil; p*, ^{32}P-labelled phosphate group.
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