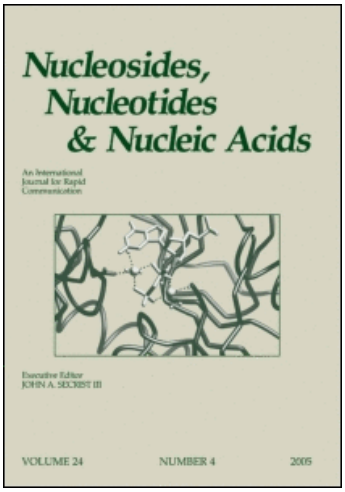

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Autoligation of Oligonucleotides via Nucleophilic Substitution Reaction

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AUTOLIGATION OF OLIGONUCLEOTIDES *VIA* NUCLEOPHILIC SUBSTITUTION REACTION

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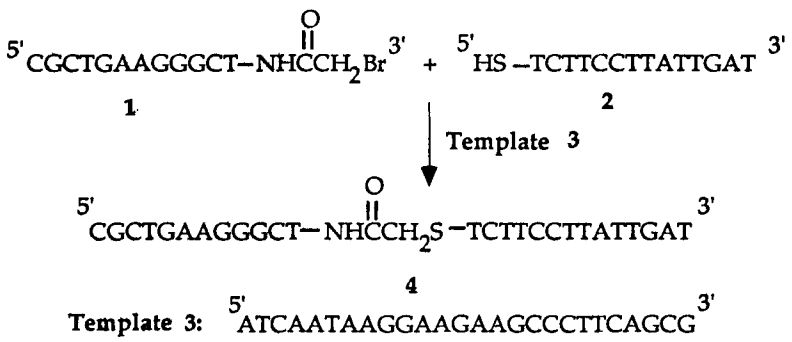
ABSTRACT: A Fast and efficient template - driven autoligation reaction between oligonucleotides derivatized with bromoacetyl and thiol groups at their opposing termini is described. The product of reaction is capable of forming a stable duplex with a complementary DNA strand.

Several methods have been reported for linking of oligonucleotides in aqueous solutions in the presence of a complementary template [1-6]. The vast majority of approaches utilize condensing or activating agents to force the reaction between terminal functional groups of oligonucleotides (phosphate - hydroxyl [1-4], phosphate - amino [2-4], phosphate - phosphate [2,5] or thiophosphate - thiophosphate [6]) to react, resulting in the formation of the ligation product. Lynn et al. describes the template - dependent coupling reaction of trimers, containing 3'-aldehydo and 5'- amino groups at opposing ends, which proceeds without condensing reagents, but requires a reducing agent to be used to stabilize the labile ligation product [7].

Recently we reported an autoligation reaction between oligonucleotides containing terminal bromoacetyl and phosphorothioate monoester groups which proceeds without a condensing agent and results in fast and efficient formation of a – $NHC(O)CH_2SP(O)^-{}_2O$ – interoligonucleoside link [8]. The ligation product is capable of forming a duplex with the complementary strand although its thermal stability is somewhat diminished compared to the phosphodiester compound, probably due to the extra three internucleoside atoms – $C(O)CH_2S$ – in the linker. Here we describe a coupling procedure, which is also based on a nucleophilic substitution reaction and results in autoligation of oligomers, derivatized with terminal bromoacetyl and thiol groups. The product of ligation contains the – $NHC(O)CH_2S$ – internucleoside linker, which has only one extra atom relative to the natural phosphodiester group.

The model system summarized in Scheme 1 was designed to examine this ligation reaction. Nucleotide sequence of the complementary ligation template 3 corresponds to the junction point of messenger RNA, derived from the *bcr-abl* (b2a2) chromosomal translocation, leading to chronic myeloid leukemia [9].



SCHEME 1

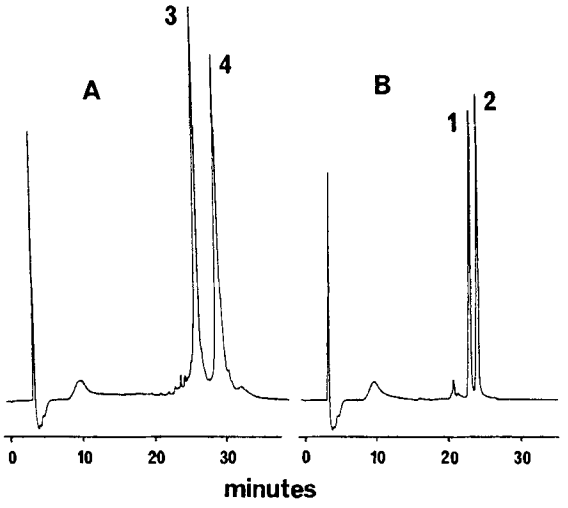


FIGURE 1. IE HPLC of products of autoligation reaction: A, oligonucleotides 1 + 2 + template 3 after 6 min; B, oligonucleotides 1 + 2 without template 3 after 6 min;
Peaks are marked according to the oligonucleotide numbers given in SCHEME 1 and TABLE 1. IE HPLC was carried out on a Dionex Omni Pak NA 100, 4x250 mm column at pH 12 (10 mM NaOH) with a 2%/min gradient of 1.5 M NaCl in 10 mM NaOH; 1 ml/min flow rate.

Equimolar amounts of the oligomers **1, 2,** and complementary template **3** (2 μM each) were mixed at room temperature in 1 ml of hybridization buffer: 0.15 mM NaCl, 10 mM Tris HCl, pH 7.02. [10] Analyses of the reaction mixture by ion exchange (IE) HPLC after 2 min and 6 min show formation of a slower eluting ligation product, oligomer **4**, with yields of about 81% and 95% respectively .

Oligonucleotide **4**, with a 3' – $NHC(O)CH_2S$ – 5' internucleoside linker, is capable of forming of a stable duplex with complementary oligonucleotide **3**, with a melting temperature, (Tm) of 66.4°C. For comparison, the Tm of the duplex formed

TABLE 1

Properties of oligonucleotides

5' Oligonucleotide 3'	R time [a] min	Tm, °C [b]
CGCTGAAGGGCT- $NHC(O)CH_2Br$; **1**	23.8	54.1
HS - TCTTCCTTATTGAT; **2**	24.9	40.8
ATCAATAAGGAAGAAGCCCTTCAGCG; **3**	26.0	-
CGCTGAAGGGCT- L- TCTTCCTTATTGAT[c]; **4**	28.4	66.4
(- S - TCTTCCTTATTGAT)$_2$; **5**	29.6	40.7
CGCTGAAGGGCTTCTTCCTTATTGAT[d] **6**	28.1	66.8

[a] Retention time on IE HPLC; conditions see legends to Figure 1.
[b] Melting temperature of the duplexes, 2μM, formed with complementary template **3** in a hybridization buffer, containing 10 mM Tris HCL , 150 mM NaCl, pH 7.02.
[c] L is a - $NHC(O)CH_2S$-linker.
[d] Oligonucleotide **6** contains only phosphodiester internucleoside linkages and possesses the same nucleotide sequence as **4**.

by the parent phosphodiester compound is 66.8°C under analogous conditions. Some characteristics of starting oligomers and reaction products are presented in TABLE 1.

It is interesting to mention that coupling of the compounds **1** and **2** on the template proceeds with about the same efficiency as the reaction between oligonucleotides dervitized with bromoacetyl and phosphorothioate monoester groups [8].

When template 3 was omitted from the reaction mixture, no ligation product **4** was observed after 6 min (FIGURE 1B). After 24 hours only about 25% of the oligonucleotide **4** was formed. Coupling without template is also accompanied by oxidative dimerization of the 5'-thiol-containing 14-mer **2**, producing approximately 15% of the 28-mer **5** (TABLE 1) containing a 5'-S-S-5' group. [11] The disulfide group of oligomer **5** does not react with the bromoacetyl function of oligonucleotide **1** in the absence or presence of template 3 as judged by IE HPLC analysis.

The presented data demonstrate the importance of the stable duplex formation for an efficient autoligation reaction. Hybridization of the oligomers **1** and **2** in contiguous mode to the same template brings strong nucleophile - 5'-thiol and electrophile - 3'-bromoacetyl in proximity, resulting in fast oligonucleotide coupling.

In summary, the described autoligation reaction could be used for oligonucleotide ligation-based diagnostics techniques, for example ligation chain reaction (LCR), as well as for antisense applications.

REFERENCES AND NOTES

1. Naylor, R., and Gilham, P.T. *Biochemistry* **1966**, 5, 2722-2728.
2. Shabarova, Z.A. *Biochemie* **1988**, 70, 1323-1334.
3. Dolinnaya, N.G., Sokolova, N.I., Gryaznova, O.I., and Shaborova, Z.A. *Nucleic Acids Research* **1988**, 16, 3721-3738.
4. Sokolova, N.I., Ashirbekova, D.T., Dolinnaya, N.G., and Shabarova, Z.A. *FEBS Letters* **1988**, 232, 153-155.
5. Purmal, A.A., Shabarova, Z.A., and Gumport, R.I. *Nucleic Acids Research* **1992**, 20, 3717-3719.
6. Gryaznov, S.M., and Letsinger, R.L. *Nucleic Acids Research* **1993**, 21, 1403-1408.
7. Goodwin, J.T., and Lynn, D.G. *J. Amer. Chem. Soc.* **1992**, 114, 9197-9198.
8. Gryaznov, S.M., and Letsinger, R.L. *J. Amer. Chem. Soc.*. **1993**, 115, 3808-3809; Gryaznov, S.M., Schultz, R., Chaturvedi, S.K., and Letsinger, R.L. *Nucleic Acids Research* **1994**, 22, 2366-2369.
9. Szcylik, C., Skorski, T., Nicolaides, N.C., Manzella, L., Malaguarnera, L., Venturrelli, D., Gewirtz, A.M., and Calabretta, B. *Science* **1991**, 253, 562-566.
10. Oligonucleotides **1** and **2** were synthesized according to procedures described in ref. 8 and in: Mag, M. Luking, S., and Engels, J.W. *Nucleic Acids Research* **1991**, 19, 1437-1441, respectively.
11. The same disulfide-containing 28-mer **5** was formed during storage of the oligomer **2** in ice at -10°C. After three weeks approximately 50% of **2** was dimerized as judged by IE HPLC; 5 can be reduced back to **2** by treatment with DTT, 1 mg/ml, 30 min. Interestingly, bromoacetyl derivative **1** did not react with DTT under these conditions.