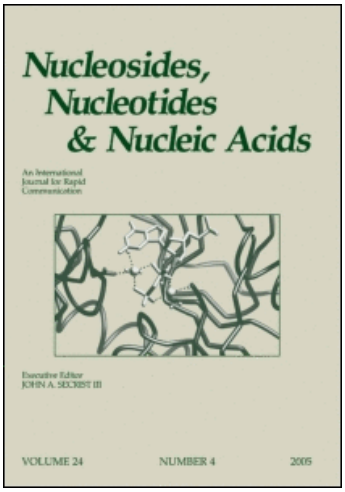

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SYNTHESIS AND PROPERTIES OF DNA DUMBBELLS CONTAINING CHEMICALLY ACTIVE SUBSTITUTED PYROPHOSPHATE INTERNUCLEOTIDE GROUPS

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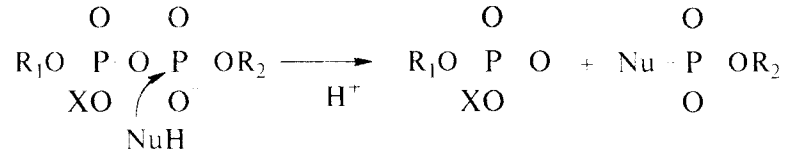
Abstract: DNA dumbbells with substituted pyrophosphate groups at a definite position of the sugar-phosphate backbone were synthesized by condensation of terminal 5'-phosphomonoester- and 3'-methylphosphodiester groups in nicked dumbbells. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide was used as a condensing agent. An efficient method for producing extended oligonucleotides carrying an O-methyl-substitued 3'-phosphate group was developed. Properties of the modified DNA-dumbbells were investigated. The substituted pyrophosphate group in the DNA dumbbells was efficiently cleaved under the action of N-methylimidazole or ethylendiamine aqueous solutions at pH 8.0.

INTRODUCTION

Synthetic analogs of DNA-recognizing proteins substrates with modifications in the sugar-phosphate backbone have already found a wide use in studies of proteins active centers topography and molecular mechanisms of DNA-protein recognition[1-4]. Synthetic DNA duplexes with a substituted pyrophosphate group in the place of the natural phosphodiester bond in a recognition site have recently been proposed as substrate analogs[5, 6]. The anhydride bond was shown to be reactive in compounds of such a structure. Thus DNA duplexes with a substituted pyrophosphate group proved to be stable in aqueous buffer solutions at pH 6.0-8.5 for several days. At the same time they

were found to interact efficiently with nucleophilic reagents, namely primary and secondary amines, including nucleopilic groups of basic amino acids[5]. The reaction proceeds by the mechanism of nucleophilic substitution at the phosphorus atom and results in the formation of a covalent bond between a part of the oligonucleotide and a nucleophilic reagent:



where R_1, R_2 are oligonucleotides, X is a nonnucleotide substitute, NuH is the nucleophilic agent.

This property led to the design of substrates capable of cross-linking with proteins. Affinity reagents were constructed for probing active centers of a set of DNA recognizing proteins. They were constructed from DNA duplexes with the substituted pyrophosphate group in a recognition site. These reagents have already been applied successfully to affinity labeling of restriction/modification enzymes *Eco*RI, RsrI[7], restriction endonuclease *Eco*RII[8] and transcription factor HNF1[9]. For example, in case of transcription factor HNF1 it has been shown that DNA substrates with the substituted pyrophosphate group can directly modify not only the active center of the protein obtained by genetic engineering but also HNF1 from a rat liver cell extract. Therefore we believe that such compounds can be used as inhibitors of a variety of enzymes and regulatory proteins, in biological studies and as potential drugs for medical treatment of a wide range of diseases, including cancer and AIDS. However, synthetic DNA duplexes have a high sensitivity to cell enzymes which greatly limits their use for these purposes. Several approaches are currently used to stabilize DNA fragments, including modifications at phosphates[10, 11] and 2'-hydroxyl groups[12, 13], formation of hairpin structures[14, 15], introduction of minihairpins with abnormally high thermostability and resistance to exonucleases at the ends of oligonucleotide[16, 17] as well as the construction of DNA dumbbells[15] and cyclic DNAs[18]. The first approach involves chemical modification of separate groups of

DNA atoms. It is a cumbersome procedure and requires an elaborate chemical synthesis and purification. We believe that the more attractive is the second method. It involves the use of hairpin DNA structures obtained by a standard synthesis on an automatic synthesizer. However Chu and Orgel[15] have shown that DNA dumbbells exhibit a much greater stability to the nuclease degradation than do DNA duplexes with a hairpin structure.

This work deals with the synthesis and properties of modified DNA dumbbells which are analogs of HNF1 transcription factor substrates and contain a chemically active substituted pyrophosphate group in the recognition site of HNF1.

MATERIALS AND METHODS

Oligonucleotides

Oligonucleotide synthesis was performed by GENSET (Paris, France), using an automated DNA synthesizer (Applied Biosystems 394/8). Oligonucleotides with the 3'-terminal phosphate group were obtained by the phosphoramidite method using the 5'-phosphorylation reagent as described in[7]. 5'-phosphate oligonucleotides were synthesized using 5'-phosphate-On cyanoethyl phosphoramidite (Clontech) as a phosphorylating reagent. 5'-end labeling of oligonucleotides was carried out by a standard procedure using T_4 polynucleotide kinase and γ^{32}P-ATP[19]. The oligonucleotides used in this study are depicted in FIG.1.

Synthesis of oligonucleotides carrying an O-methyl-substituted 3'-phosphate group (3'-O-methyl esters).

The synthesis of 3'-O-methyl esters of the oligonucleotides was performed as following. The 3'-phosphorylated oligonucleotides (0.001-0.02 μmol) were incubated for 12 h at 4°C in 110 μl of a medium containing 0.25M MES, pH 4.5, 0.5M $MgCl_2$, 35% CH_3OH and 11 mg (57.4 μmol) of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDAC). Following the incubation, the oligonucleotides were recovered by precipitation with 10 volumes of 2%

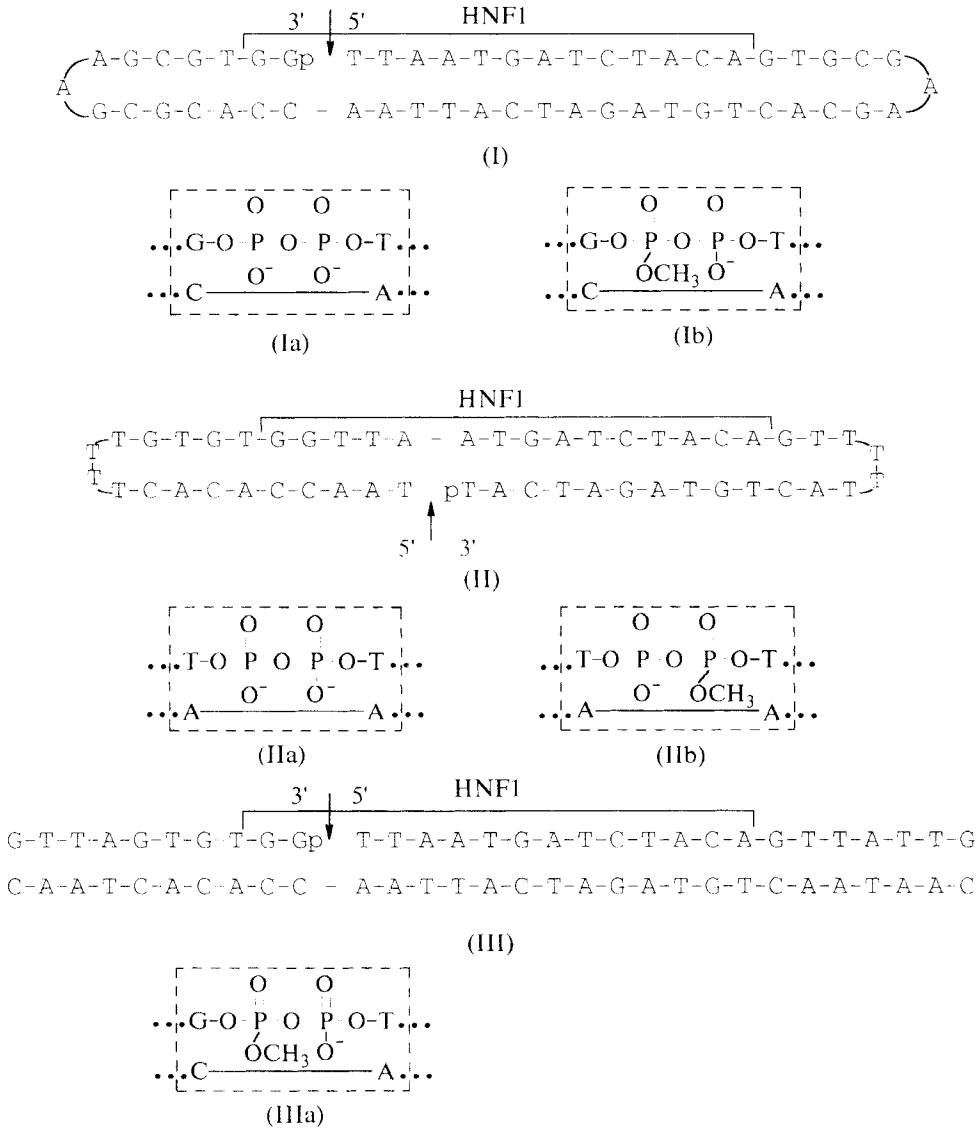


FIG. 1. Sequences of deoxyoligonucleotides used in this work.
Horizontal lines indicate the recognition site of HNF1; arrows mark the site of the oligonucleotide junction; dashed lines mark diagrammic representation of the modified DNA dumbbells and DNA duplex (IIIa) where modified fragments and nucleotides facing the nick are indicated. For sequences (Ia) and (Ib) see (I), for sequences (IIa) and (IIb) see (II), for sequence (IIIa) see (III).

$LiClO_4$ in acetone and further reprecipitated three times by resuspension in 2M $LiClO_4$ and addition of 10 volumes of acetone. The 3'-O-methyl esters of the oligonucleotides were isolated by reverse-phase HPLC on a Delta Pak 300 Å column (3,9x150 mm, particle size 7 μ), using a linear gradient of acetonitrile in 0.1 M triethylammonium acetate buffer, pH 7.0.

After the isolation the 3'-O-methyl esters of the oligonucleotides were desalted by multiple evaporation to dryness from 50% ethanol at 50°C.

Synthesis of DNA dumbbells containing the substituted pyrophosphate group

A mixture of the 5'-P- and 5'-^{32}P-phosphorylated 3'-O-methyl esters of the oligonucleotides (oligonucleotide concentration per monomer was 10^{-3} M) in 0.05 M MES-buffer, pH 6.0, 0.02 M $MgCl_2$ was incubated at 95°C for 2 min and slowly cooled for several hours. Then EDAC was added to the concentration of 0.2M. The reaction was carried out in the dark for 16 h at 4°C. The oligonucleotide material was precipitated with acetone, the reaction mixture was analyzed by electrophoresis in 15% polyacrylamide gel (PAGE). Reaction products were eluted from the gel with 2 M $LiClO_4$, precipitated with 5 volumes of acetone and reprecipitated by resuspention in 2 M $LiClO_4$ and addition of 10 volumes of acetone.

Synthesis of DNA dumbbells containing the pyrophosphate group

Synthesis of DNA dumbbells with a pyrophosphate group was accomplished in conditions of the synthesis of the DNA dumbbells with the substituted pyrophosphate group using 5'- and 3'-phosphorylated nicked DNA dumbbells (I) and (II) as described above.

Analysis of the modified DNA dumbbells with the pyrophosphate or substituted pyrophosphate groups

Treatment with a mixture of phosphodiesterase and phosphatase

The modified DNA dumbbells (0.1-0.5 nmol) dissolved in 10-30 μl of 0.2M Tris-HCl buffer, pH 8.5, 0.04M $MgCl_2$, were treated with a mixture of alkaline phosphatase and snake's venom phosphodiesterase for 3 h at 37°C. The oligonucleotide material was precipitated with a 2% LiClO4 solution in acetone

and analyzed by denaturing 15% PAGE. 0.05M Tris-borate buffer, pH 8.5, 0.001M EDTA was used for PAGE.

Treatment with N-methylimidazole (N-MeZm) or ethylendiamine (EDA)

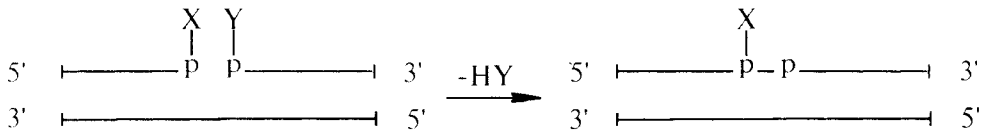
The DNA dumbbells (0.1-0.5 nmol) were treated with a 0.4 M aqueous solution of N-MeIm, pH 8.0, or a 0.5 M aqueous solution of EDA, pH 8.0, for 16 h at 50°C. The oligonucleotide material was precipitated with 2% $LiClO_4$ in acetone, reprecipitated two times and analyzed by 15% PAGE.

N-MeIm treatment of a linear DNA duplex with the substituted pyrophosphate group

DNA duplex IIIa (0.2 nmol) was treated with the 0.4 M aqueous solution of N-MeIm pH 8.0, for 16 h at 37° and 50°C. The reaction mixture was precipitated as described for the DNA dumbbells and analyzed by 20% PAGE.

RESULTS AND DISCUSSION

So far approaches have been developed to obtain cyclic DNA, including dumbbells, both by enzymatic methods using DNA-ligase[11, 20] and by the chemical ligation with the aid of different condensing agents, namely aqueous soluble carbodiimide[18, 21], N-methylimidazole and N-hydroxybenzotriazole[18]. These approaches were used to synthesize a set of cyclic DNAs with the phosphodiester[18, 20-22] or pyrophosphate[18] internucleotide bond at the ligation site. For the synthesis of DNA dumbbells with the substituted pyrophosphate group, chemical ligation under the action of EDAC was utilized. We have shown before[5] that this method was the best for the introduction of a substituted pyrophosphate group into linear DNA duplexes. The approach involves condensation of two oligonucleotides on a complementary template. One of those oligonucleotides has a residue of an aliphatic alcohol or an amine at its 3'-end phosphate group, the other has an activated 5'-end phosphate group:



where straight lines designate oligonucleotides, X are residues of an aliphatic alcohol or an amine, Y is the residue of EDAC.

The efficiency of ligation varied in the range from 35 to 80% being dependent on the length and structure of the DNA duplex as well as on the nature of the nonnucleotide substituent[5].

50- and 52-membered oligonucleotides were selected as initial oligonucleotides. They form the following structures in a aqueous buffer solution (FIG. 1). Initial nicked DNA dumbbells (I) and (II) contained the recognition site of the transcription factor HNF1, flanked on both sides by minihairpin structures 5' GCGAAGC 3' enhancing stability of the initial duplex[16] (dumbbell I) or by tetranucleotide sequences T_4 (dumbbell II). In dumbbell (I) the substituted pyrophosphate group was introduced between nucleotides G and T of the upper chain of the recognition site since it was shown earlier that the interaction of HNF1 with a similar modified linear DNA duplex leads to cross-linking[9]. In dumbbell (II) the substituted pyrophosphate group was inserted between nucleotides T and T of the lower chain of the recognition site.

We used a methoxy group as the nonnucleotide substituent. We have found before that the cleavage of a substituted pyrophosphate bond is faster when the nonnucleotide substitute X is an alcoxy group[23]. Consequently, the cross-linking must also be more efficient in this case. The methoxy group was selected also for it had the lowest distorting effect on DNA structure as compared to other alcoxy groups. Initial oligonucleotides which formed nicked DNA dumbbells (I) and (II) were synthesized by the standard phosphoramidite method (see MATERIALS AND METHODS).

The next stage involved methylation of the 3'-end phosphate group of compounds (I) and (II). It was carried out in an aqueous buffer solution with excess of methanol under the action of EDAC. The reaction of the terminal phosphate group alkylation in oligonucleotides was developed by Ivanovskaya and coauthors[24]. The reaction routinely proceeds with a quantitative yield within 4-5-hours. However we have shown that this is the case only for oligonucleotides up to 15-17-mers. Methylation of phosphate group of more

extended oligonucleotides following the standard turned out to be inefficient. The point is that addition of methanol to a salt solution of an oligonucleotide (methylation buffer contains 1 M $MgCl_2$) is a classical strategy for precipitating extended oligonucleotides but it fails to precipitate short oligonucleotides. That is why a major portion of the oligonucleotide material precipitates, the amount of precipitate depending on the oligonucleotide concentration. To avoid the precipitation we slightly changed the methylation procedure and adjusted it for extended (up to 52-mer) oligonucleotides. By decreasing methanol amount from 50% to 35% and increasing the reaction time to 12 h, we managed to achieve a quantitative transformation of 3'-phosphorylated oligonucleotides forming nicked DNA dumbbells (I) and (II) into corresponding methyl esters (FIG.2).

The substituted pyrophosphate group was introduced into the sugar-phosphate backbone of the 3'-O-methyl esters of DNA dumbbells (I) and (II) by condensation of their 5'-phosphomonoester (for 5'-phosphorylation see MATERIALS AND METHODS) and 3'-methylphosphodiester groups under the action of EDAC. The reaction was carried out in 0.05M MES, pH 6.0, containing 0.02 M $MgCl_2$, at 4°C for 16 h. Similar dumbbells were synthesized as a control. They contained a nonsubstituted pyrophosphate group at the same sites. This group, unlike the substituted pyrophosphate group, is exceptionally stable and get cleaved by trifluoroacetic anhydride under severe conditions[25].

The nonsubstituted pyrophosphate group was synthesized by condensation of the 3'- and 5'-end phosphate groups of DNA dumbbells (I) and (II) under the action of EDAC. FIG.3 presents an autoradiogram of the reaction mixtures after the synthesis of DNA dumbbells (Ia) and (Ib). In both cases chemical ligation led to products with a higher electrophoretic mobility than that of the initial compounds. The yields were 25 and 20%, respectively. A similar change in the electrophoretic mobility was observed during the synthesis of the natural phosphodiester bond in DNA dumbbells with different base sequence and chain length[21]. Synthesis of DNA dumbbells (IIa) and (IIb) followed a similar pattern. However the yields of chemical ligation were higher, 45 and 25% respectively.

We attribute the difference in the yields of chemical ligation products in dumbbells (I) and (II) to different distances between the sites of the internucleotide bond synthesis and the loops as well as to different nature of nucleotides to be joined[26].

Yields of DNA dumbbells with the nonsubstituted pyrophosphate group are higher than yields of analogous DNA dumbbells with the substituted pyrophosphate group. This may be attributed to the different nucleophilicity of mono- and disubstituted phosphate groups involved in the reaction.

DNA dumbbells (Ia), (Ib), (IIa) and (IIb) synthesized by chemical ligation proved to be stable to the action of snake venom phosphodiesterase and alkaline phosphatase. This confirms their cyclic structure. It was of special interest to study the reactivity of the substituted pyrophosphate group incorporated into the DNA dumbbells since the anhydride bond in trisubstituted pyrophosphates showed different activity in compounds of different nature. Thus the substituted pyrophosphate group incorporated into single-stranded oligonucleotides is stable enough in aqueous buffer solutions at pH 6.0-8.5. At the same time it is readily and quantitatively cleaved within several hours at 37°C by a 0.5M aqueous solution of EDA, pH 8.0, or by 0.4M solution of N-MeIm, pH 8.0[5]. Linear 13-14-membered DNA duplexes with the substituted pyrophosphate group proved to be more stable than single-stranded oligonucleotides [8].

The reactivity of DNA dumbbells (Ib) and (IIb) with a substituted pyrophosphate group was studied using EDA and N-MeIm as nucleophilic reagents. As a control we took an analogous linear 30-membered DNA duplex (IIIa) containing the substituted pyrophosphate group between nucleotides G and T (see FIG. 1) and DNA dumbbells (Ia) and (IIa) with the nonsubstituted pyrophosphate group. DNA duplex (IIIa) was synthesized as described in[5]. An autoradiogram of the reaction mixtures after aminolysis of compounds (Ia), (Ib) and (IIIa) by N-MeIm is shown on FIG. 4. Aminolysis of linear DNA duplex (IIIa) was carried out at 37°C and 50°C, aminolyses of the rest of the compounds were performed at 50°C for 16 h. It is evident from the FIG. 4. that DNA dumbbell (Ia) with the nonsubstituted pyrophosphate bond is perfectly

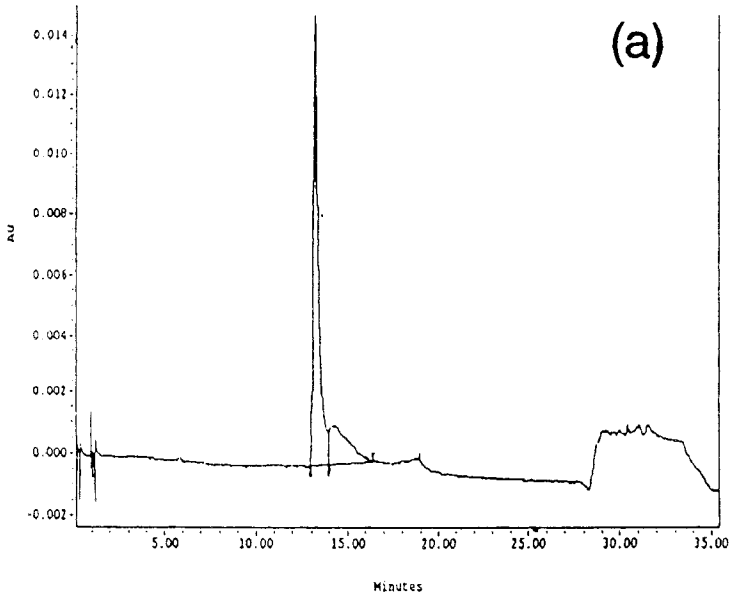


FIG. 2. HPLC elution profiles of a) initial deoxyoligonucleotide (I), b) the reaction mixture resulting from the synthesis of the 3'-O-methyl ester of deoxyoligonucleotide (I), c) the mixture of a) and b). Separation conditions see in MATERIALS AND METHODS.

stable under these conditions. Yet the efficacy of cleavage of the substituted pyrophosphate group incorporated into DNA duplex (IIIa) is 90% at 50°C and only 50% at 37°C. The efficacy of cleavage of the modified group in DNA dumbbell (Ib) is 40% at 50°C, i.e. the anhydride bond is more stable in this case. An autoradiogram of the reaction mixtures formed by EDA treatment of compounds (Ia) and (Ib) at 50°C is given on FIG. 5. As one can see on the figure, aminolysis of compound (Ib) is also about 40%. In this case aminolysis produces aminoethylamide of initial nicked DNA duplex (I). This was confirmed by acid hydrolysis the phosphoamidate bond in this compound with

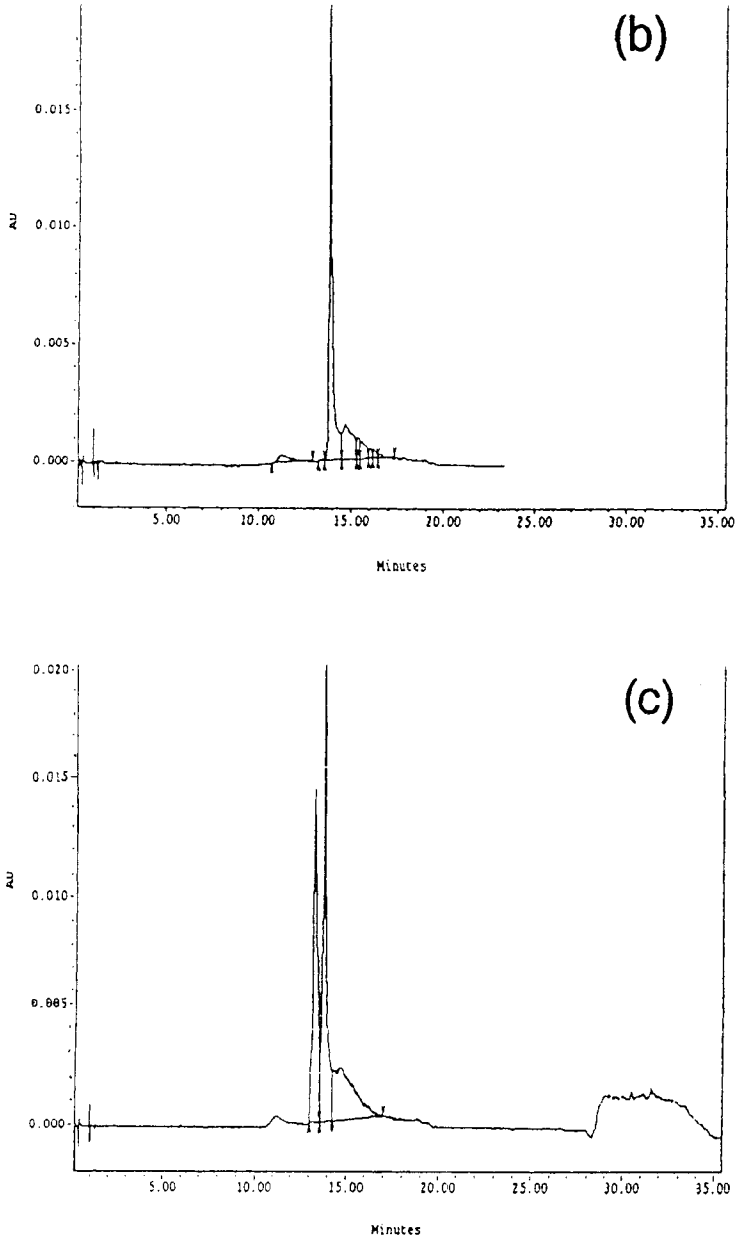


Fig. 2. Continued

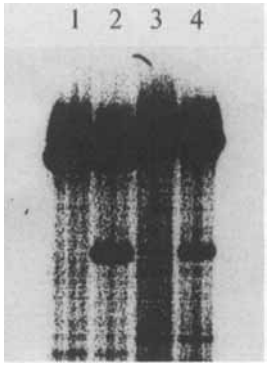


FIG.3. Electrophoretic analysis of the reaction mixtures after the synthesis of modified DNA dumbbells with pyrophosphate (Ia) and substituted pyrophosphate (Ib) bonds. 1 and 3, nicked DNA dumbbells (Ia) and (Ib); 2 and 4, reaction mixtures after the synthesis of (Ia) and (Ib). For structures see FIG. 1; for conditions see MATERIALS AND METHODS. The ^{32}P-label was introduced into the 5'-end of deoxyoligonucleotides.

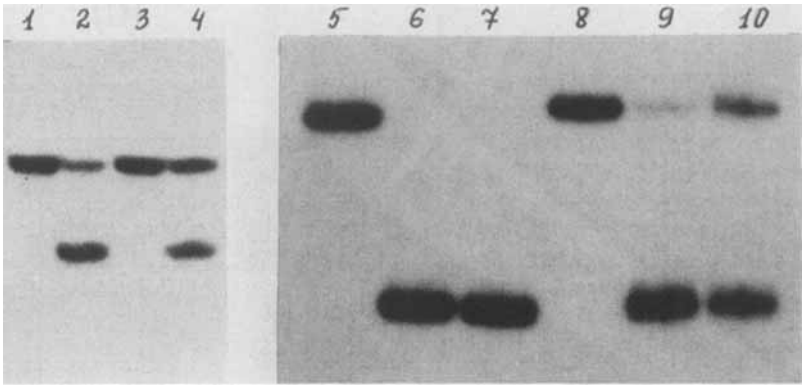


FIG.4. Electrophoretic analysis of the reaction mixtures resulting from the 0.4 M N-MeIm treatment of DNA dumbbells(Ia), (Ib) and DNA duplex (IIIa). 1 and 3, DNA duplex (IIIa); 2 and 4, the reaction mixtures resulting from the treatment of DNA duplex (IIIa) at 50°C and 37°C; 5 and 8, nicked DNA dumbbells (Ia) and (Ib); 6 and 9, DNA dumbbells (Ia) and (Ib); 7 and 10, the reaction mixtures resulting from the treatment of DNA dumbbells (Ia) and (Ib) at 50°C. For conditions see MATERIALS AND METHOD. The ^{32}P-label was introduced into disubstituted phosphate of substituted or nonsubstituted pyrophosphate groups of the modified compounds.

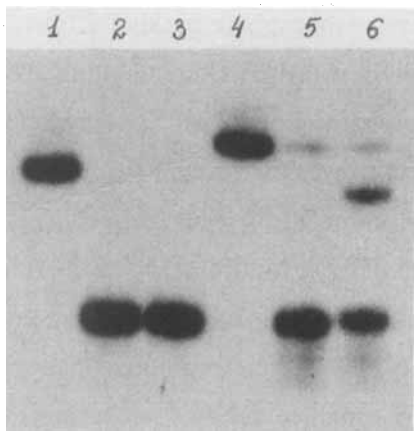


FIG.5. Electrophoretic analysis of the reaction mixtures resulting from 0,5 M EDA treatment of DNA dumbbells (Ia) and (Ib). 1 and 4, nicked DNA dumbbells (Ia) and (Ib); 2 and 5, DNA dumbbells (Ia) and (Ib); 3 and 6, the reaction mixtures resulting from the treatment of (Ia) and (Ib). For conditions see MATERIALS AND METHODS.

50% acetic acid for 2 h at 50°C. Similar results were obtained for compounds (IIa) and (IIb). The data obtained attest that the substituted pyrophosphate group in DNA dumbbells is chemically active and interacts with nucleophilic agents in aqueous media at pH 8.0. However its reactivity is less than that of the substituted pyrophosphate group incorporated into linear DNA-duplexes and especially than the reactivity of that group incorporated into single-stranded oligonucleotides. This may be associated with extra stability of such compounds due to the formation of a dumbbell structure and therefore with less accessibility of the disubstituted phosphorus atom for a nucleophilic attack.

Hence, on the one hand, DNA dumbbells with a substituted pyrophosphate group are stable to cell exonucleases and on the other hand, they are capable of efficient interacting with nucleophilic agents in aqueous media. Thus DNA dumbbells with the substituted pyrophosphate group in the site of recognition of DNA binding proteins are promising reagents for probing active centers of these proteins and for revealing nucleophilic amino acids contacting

directly with the DNA sugar-phosphate backbone. They also may be promising inhibitors of protein factors. Furthermore, we think that DNA dumbbells with nonsubstituted pyrophosphate groups can be used for probing electrostatic DNA-protein contacts and revealing protein fragments in the active center that are rich in nucleophilic amino acids. This suggestion is based on the fact that introduction of this modification into the DNA sugar-phosphate backbone gives rise to extra negative charge which in turn may lead to enhanced binding of such analogs to positively charged nucleophilic amino acids.

We plan to use the modified DNA dumbbells synthesized in this work in HNF1 transcription factor studies.

REFERENCES

1. Guga, P., Koziolkiewicz, M., Okruszek, A., Uznanski, B. and Stec, W.J. Nucleosides and Nucleotides, 1987, 6, 111.
2. Olsen, D.B., Kotzorek, G., Sayers, J.R. and Eckstein, F. J. Biol. Chem., 1990, 265, 14389.
3. Koziolkiewicz, M. and Stec, W.J. Biochemistry, 1992, 31, 9460.
4. Mayer, A.N. and Barany, F. J. Biol. Chem., 1994, 269, 29067.
5. Kuznetsova, S.A., Ivanovskaya, M.G. and Shabarova, Z.A. Bioorgan. chimia (Russ.) 1990, 16, 219.
6. Kuznetsova, S.A., Ivanovskaya, M.G. and Shabarova, Z.A. Nucleic Acids Res., 1991, Symp. Ser., 235.
7. Purmal, A.A., Shabarova, Z.A. and Gumport, R.I. Nucleic Acids Res., 1992, 20, 3713.
8. Shabarova, Z.A., Sheflyan, G.Ya., Kuznetsova, S.A., Kubareva, E.A., Sysoev, O.N., Ivanovskaya, M.G. and Gromova, E.S. Bioorgan. Khimia (Russ.) 1994, 20, 413.
9. Blumenfeld, M., Kuznetsova, S., Clusel, C., Ugarto, E., Shabarova, Z. and Vasseur, M. J. of Cell. Biochemistry, 1994, 186, 42.
10. Tam, R.C., Li, Y., Noonberg, S., Hwang, D.G., Lui, G., Hunt, C.A. and Garovoy M.R., Nucleic Acids Res., 1994, 22, 977.

11.Fathi, R., Huang, Q., Coppola, G., Delaney, W., Teasdale, R., Krieg, A.M. and Cook, A.F. Nucleic Acids Res., 1994, 22, 5416.

12.Sproat, B.S., Lamond, A.I., Beijer, B., Neuner, P. and Ryder, U. Nucleic Acids Res., 1989, 17, 3373.

13.Iribarren, A.M., Sproat, B.S., Neuner, P., Sulston, I., Ryder, U. and Lamond, A.I. Proc. Natl. Acad. Sci. USA, 1990, 87, 7747.

14.Chu, B.C.F. and Orgel, L.E. Nucleic Acids Res., 1991, 19, 6958.

15.Chu, B.C.F. and Orgel, L.E. Nucleic Acids Res., 1992, 20, 5857.

16.Hirao I., Kawai, G., Yoshizawa, S., Nishimura, Y., Ishido, Y., Watanabe, K. and Miura, K. Nucleic Acids Res., 1994, 22, 576.

17.Khan, I.M. and Coulson J.M. Nucleic Acids Res., 1993, 21, 2957.

18.Dolinnaya, N.G., Blumenfeld, M., Merenkova, I.N., Oretskaya, T.S., Krynetskaya, N.F., Ivanovskaya, M.G., Vasseur, M. and Shabarova, Z.A. Nucleic Acids Res., 1993, 21, 5403.

19.Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley - Interscience, 1989, New York.

20.Wemmer, D.E. and Benight, A.C. Nucleic Acids Res., 1985, 13, 8611.

21.Ashley, G.W. and Kushlan, D.M. Biochemistry, 1991, 30, 2927.

22.Erie, D.A., Jones, R.A., Olson, W.K., Sinha, N.K. and Breslauer, K.J. Biochemistry, 1989, 28, 268.

23.Kuznetsova. S.A., Kanevsky, I.E., Florent'ev, V.L., Mirzabekov, A.D. and Shabarova, Z.A. Molecular Biology (Russ.) 1994, 28, 190.

24.Ivanovskaya, M.G., Gottikh, M.B. and Shabarova, Z.A. Nucleosides and Nucleotides, 1987, 6, 913.

25.Purmal, A.A., Drutsa, V.L. and Shabarova, Z.A. Bioorgan. Khimia (Russ.) 1988, 14, 1183.

26. Dolinnaya, N.G., Merenkova, I.N., and Shabarova, Z.A. Nucleosides and Nucleotides, 1994, 13, 2169.

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