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Polyamine-linked oligonucleotides for DNA triple helix formation

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ABSTRACT

The concept of antigene therapy of disease is based on the ability of an oligonucleotide (the therapeutic agent) to bind to double-stranded genomic DNA (the target associated with the disease). Examples are herein given of the linkage of a series of polyamines to a 21-mer homopyrimidine oligonucleotide. These conjugated 21-mers can each form a triple helix with an appropriate double-stranded homopurine-homopyrimidine DNA according to Hoogsteen base-pairing rules. No triple helix was found when unmodified third strand was used at 10 mM sodium phosphate, pH 6.5, 100 mM sodium chloride solution. In contrast, the spermine-conjugated oligonucleotide had a melting temperature of 42°C. According to the melting profile, the appended spermine moiety was found to affect the T_m only of the triple helix, but not of the subsequent melting of the underlying double helix. The T_m enhancing ability of the spermine-conjugate was found to be better than that of other polyamine-conjugates.

INTRODUCTION

The application of short oligonucleotides for control of gene expression could become a new type of gene therapy (1,2). Antigene oligonucleotides have been designed to interact with specific regions on double-stranded genomic DNA to inhibit transcription. For example, the intracellular level of c-myc mRNA was reduced by using a triple helix-forming oligonucleotide that binds to the c-myc promoter (3). HIV-1 transcription was inhibited by triple helix formation at the transcription initiation site and the nuclear factor S1 binding site (4).

In one common motif, the third (i.e. antigene) strand contains only thymine and cytosine (i.e. a homopyrimidine strand). The third strand binds to double-stranded DNA via Hoogsteen hydrogen bonds, in which T base pairs with the A of an A-T pair and (protonated) CH^+ pairs with the G of a G-C pair. Since the cytosine has to be protonated to form two hydrogen bonds with guanosine, this type of triplex was found to be more stable at acidic pH, compared with neutral or basic pH. Cytosine methylated at the 5′ position also has been used for triple helix formation, since it remains protonated at a higher pH (5). Analogs of protonated cytosine have been incorporated into third strands for increased binding ability at neutral or basic pH (6–11), thereby making these systems more consistent with *in vivo* conditions.

Certain DNA binding compounds can stabilize triple helices. These include benzo[ϵ]pyridoindole derivative which is a triple helix specific intercalator (12), and spermine (13). In each case, the stabilizer is added separately, rather than being covalently attached to the triplex-forming strand. Acridine, a double-stranded DNA intercalator has been covalently linked to the end of the third strand, and found to increase the melting temperature of the third strand (14). Psoralen, not only an intercalator but also a photoactivatable cross-linking compound, was attached to the terminus of the triple helix-forming strand (15, 16). Compounds such as acridine and psoralen, however, may not be suitable in a therapeutic product due to potential toxicity concerns.

Polyamines have been reported to stabilize double-stranded DNA when present at μM levels (17). Based on the 2.0 A crystal structure, it was found that spermine binds to the deep major groove of A-form duplex DNA (18). Spermine has been widely used for stabilizing triple helix DNA, but at higher concentrations than those needed for stabilizing double-stranded DNA. It has been reported that a homopyrimidine-homopurine-homopyrimidine triple helix can be stabilized by low mM levels of spermine (13) at physiologic pH and temperature. From fiber diffraction studies (19), it was suggested that the third oligonucleotide strand binds to the deep major groove of the A-form of double-stranded DNA. However, recent studies by NMR (20), fourier transform infrared spectroscopy (21) and molecular modeling (22) indicate preference for the B-form structure. All studies, however, agree that the third strand locks into the major-groove of the duplex DNA. Thus, spermine as well as third strand oligonucleotides might both bind to the major-groove. Based on these results, we decided to covalently link spermine and other polyamines to the terminus of an oligonucleotide in order to investigate the possibility of a third strand conjugate with superior triple helix-forming properties.

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EXPERIMENTAL

Oligonucleotides, including those with a 5'-hexanolamine group, were synthesized by cyanoethyl phosphoramidite chemistry using an automatic DNA synthesizer (380B, ABI, Foster City, CA), and purified by anion-exchange chromatography on a Nucleogen DEAE column. The HPLC system consisted of two Beckman (San Ramon, CA) 114M pumps, a 421A controller and a Linear 200 UV detector.

Preparation of trityl mercaptopropionic acid

Triphenylmethanol (2.6 g, 10 mmol) and mercaptopropionic acid (870 μl, 10 mmol) were mixed in acetic acid (20 ml) at 70°C. Boron trifluoride etherate (147 μl, 12 mmol) was added to the previous solution. The reaction mixture was kept at 70°C for 30 min, then stirred at room temperature for another 30 min. The product was precipitated when the reaction mixture was poured into water (100 ml). The precipitate was collected and washed extensively with water; 3.40 g was obtained (98% yield). NMR(DMSO-d_6), δ=2.16(t, 2H), 2.28(t, 2H), 7.32(s, 15H), 12.22(br, 1H).

Preparation of trityl mercaptopropionyl-(N-methoxy-N-methyl) carboxamide

The synthetic procedure was modified from Fehrentz and Castro (23). Trityl mercaptopropionic acid (696 mg, 2 mmol), benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent, 696 mg, 2 mmol) and triethyl amine (300 μl) were mixed in methylene chloride (10 ml). N,O-dimethylhydroxylamine hydrochloride (214 mg, 2.2 mmol) and triethylamine (300 μl) were premixed in methylene chloride (10 ml) in another flask. Thirty minutes later, both solutions were mixed and stirred at room temperature for another hour. The reaction mixture was washed with hydrochloric acid solution (1 M, 2×50 ml), saturated sodium chloride solution (2×50 ml), 1 M sodium bicarbonate solution (2×50 ml) and saturated sodium chloride solution (2×50 ml). The organic solvent was removed under vacuum and purified by chromatography (silica gel 60−200 μm); 581 mg was obtained (74% yield). NMR(DMSO-d_6), δ=2.32(m, 4H), 3.01(s, 3H), 3.53(s, 3H), 7.34(s, 15H).

Preparation of trityl mercaptopropionyl aldehyde

Lithium aluminum hydride (50 mg, 1.3 mmol) was added to the solution of trityl mercaptopropionyl-(N-methoxy-N-methyl) carboxamide (391 mg, 1 mmol) in ethyl ether (10 ml). Twenty minutes later, a solution of potassium hydrogen sulfate (477 mg, 3.5 mmol) in water (10 ml) was poured into the reaction mixture. The ethyl ether layer was washed with 1 M hydrochloric acid (2×25 ml), saturated sodium chloride solution (2×25 ml), 1 M sodium bicarbonate (2×25 ml) and saturated sodium chloride solution (2×25 ml). The organic layer was dried under vacuum. The oily residue (311 mg) was used in the next step without any further purification.

Preparation of trityl mercaptopropyl spermine

Crude trityl mercaptopropionyl aldehyde (311 mg, <0.9 mmol) in methanol (5 ml) was added dropwise to the solution of spermine (202 mg, 1 mmol) and sodium cyanoborohydride (31 mg, 0.5 mmol) in methanol (10 ml). Thereafter, acetic acid (100 μl) was added to the reaction mixture. The reaction was complete within 30 min. Unreacted sodium cyanoborohydride was hydrolyzed by water (5 ml). The product was purified by reverse phase HPLC on a Vydax C-18, 10 μm column (25×1.0 cm). Mobile phase A was 0.1% trifluoroacetic acid in water and mobile phase B contained 70% acetonitrile, 20 % isopropanol and 10% mobile phase A. The flow rate was 3 ml/min. The gradient was 70% A to 0% A in 50 min. The desired product eluted at 33 min. NMR(DMSO-d_6), δ=1.60(m, 6H), 1.88(m, 4H), 2.16(t, 2H), 2.89(m, 14H), 7.30(s, 15H). FAB-MS $[M+H]^+$=518.

Preparation of oligonucleotide-spermine conjugate (3,4,3-Z)

Iodoacetylated oligonucleotide was prepared as reported (24). Amino-linked oligonucleotide (5 units A_{260}) and N-hydroxysuccinimide iodoacetic ester (6 mg) (25) were reacted in dimethylformamide/0.1 M sodium bicarbonate solution (500 μl, 2:3) for two hours. After the reaction, the oligonucleotide was recovered from the reaction mixture by ion-exchange chromatography (26), on a Nucleogen 60-7 DEAE column (125×4 mm). Mobile phase A was 95% 0.1 M triethylammonium acetate, pH 7.0, and 5% acetonitrile. Mobile phase B was mobile phase A containing 0.7 M lithium chloride. The gradient was 100% isocratic A for 5 min, 100% A to 88 %A for 25 min, and 88% A to 0% A in 5 min. The eluent at 36 min was collected.

Separately, trityl mercaptopropyl spermine (1 mg) was treated with trifluroacetic acid (100 μl) for 5 min, then dried under vacuum. The iodoacetylated oligonucleotide, as previously collected from the ion exchange column, was adjusted to a 4 M guanidine hydrochloride solution by adding solid guanidine hydrochloride. The oligonucleotide solution was poured into the tube of mercaptopropionyl spermine and adjusted to about pH 7.6−8.0 with 1 M sodium bicarbonate solution. The reaction mixture was shaken at room temperature overnight.

The excess spermine derivative was removed by gel-permeation chromatography on a Bio-Gel TSK-30 (300×7.5 mm) column. The mobile phase was 2 M sodium chloride. Flow rate was 0.5 ml/min. The eluent from 14−18 min was collected.

The collected portion from the gel-permeation chromatography was purified by reverse phase chromatography on a PRP-1 column (10 μm, 150×4.1 mm, Hamilton). The flow rate was 1 ml/min. Mobile phase A was 95% 100 mM triethylammonium acetate, pH 8.0, and 5% acetonitrile, and mobile phase B was

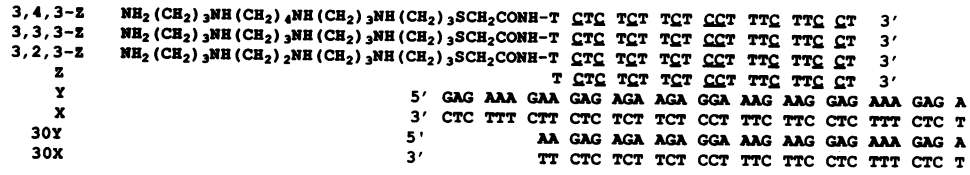

Figure 1. Structures, sequences and abbreviations of oligonucleotides used in this study. The underlined C on the Z and its conjugate strands is 5-methylated cytosine.

95% acetonitrile and 5% 100 mM triethylammonium acetate, pH 8.0. The gradient was 100% A for 5 min, then 100% A to 85% A in 45 min. The peak at 34 min was collected. The overall yield was 19%.

N,N'-Bis(3-aminopropyl)-1,3-propanediamine-oligonucleotide and N,N'-Bis(3-aminopropyl)ethylenediamine-oligonucleotide (referred to as 3,3,3-Z and 3,2,3-Z, respectively) were synthesized using the same procedure.

Melting curves

UV melting profiles were obtained at 260 nm at a heating rate of 0.5°C/min using a computer-interfaced Perkin-Elmer model 575 spectrophotometer equipped with a thermoelectrically controlled cell holder. The solution was 10 mM sodium phosphate buffer, pH 6.0–7.5, 100 mM sodium chloride. The concentrations of strands X, Y and Z were 1 μM, 1 μM and 2 μM, respectively. The mixture was heated at 90°C for 15 min, then slowly cooled to room temperature and kept overnight. The precision of T_m measurement was considered to be within 1°C.

CD transition curves at 218 nm were obtained using an Aviv model 60DS spetropolarimeter (Lakewood, NJ) equipped with a thermoelectrically controlled cell holder. The same solution for the UV melting experiment was used for CD.

RESULTS AND DISCUSSION

Design of the oligonucleotides

The sequences of the three strands used in this study were obtained from Hanvey (13). To provide space on either end for polycation binding in the major groove, strands X and Y, which form the double-stranded target, were extended in both directions to 37-mers (Figure 1). Thus, upon binding, the third strand (the 21-mer strand Z) will be centered on the double-stranded target, with an overhang of 8 base pairs on either side to accomodate a polyamine appended at the 3' or the 5', or even both termini. In addition, 5-methylated cytosine was used in place of cytosine in the third strand to enhance triple helix formation (5, 27). In this initial study, a hexanolamine liker was attached to the 5' end of the third strand for conjugation to the polyamine.

Preparation of oligonucleotide-polyamine conjugates

Spermine has clearly been demonstrated to promote triple helix formation at near physiologic conditions (13). In contrast, spermidine, which has one alkylamino group less than spermine, was shown to have no effect on triple helix stabilization (13). Thus, the number of amino groups seems to be critical to triple helix formation. To preserve all charged amino groups on the polyamine, an amide linkage to the polyamine was unacceptable. Therefore, the procedure of forming and reducing a Schiff base into a secondary amine was used (Figure 2).

In the reaction scheme of Figure 2, a thiol adduct of the polyamine was prepared for selective coupling to the oligonucleotide. As mentioned above, this thiol group was appended to the polyamine in such a manner as to preserve the four positive charges, as well as to avoid a branched structure from one of the central secondary amines. Since the free thiol group itself may form a disulfide bond, it was protected by a trityl group from the beginning. The trityl protecting group was removed prior to the final conjugating reaction.

Conversely, an iodoacetyl group was appended to the 5' terminus of the oligonucleotide for selective coupling to the thiol group in the polyamine. An amino-linked oligonucleotide, prepared by standard protocols on a DNA synthesizer, was reacted with iodoacetic acid-activated ester in basic 40% dimethylformamide solution. More than 95% of the oligonucleotide was acetylated under this condition, according to the HPLC analysis (data not shown). On chromatographic purification, oligonucleotides were bound to an anion-exchange column, whereas all the excess reagents, byproducts and dimethylformamide were removed in one step. The iodo-activated oligonucleotide was eluted with increasing salt concentration and then used immediately for coupling to the activated spermine.

When a large excess of polyamine is mixed with an oligonucleotide, a precipitate or aggregate can form due to charge interactions. Accordingly, a high salt concentration was always used to interfere with these charge interactions. Furthermore, excess polyamine derivative was removed by gel-permeation chromatography before applying to the reverse-phase chromatography purification. In reverse-phase chromatography, the conjugate eluted slightly later than the starting amino-linked oligonucleotide (Figure 3). Compared to the oligonucleotide, the conjugate had a lower net negative charge and migrated more slowly in gel electrophoresis (data not shown).

Spermine contribution to triple helix stability

Under appropriate conditions and with an appropriately designed third strand, the UV melting profile of a triple helix, may exhibit two resolved transitions (27). The first transition corresponds to

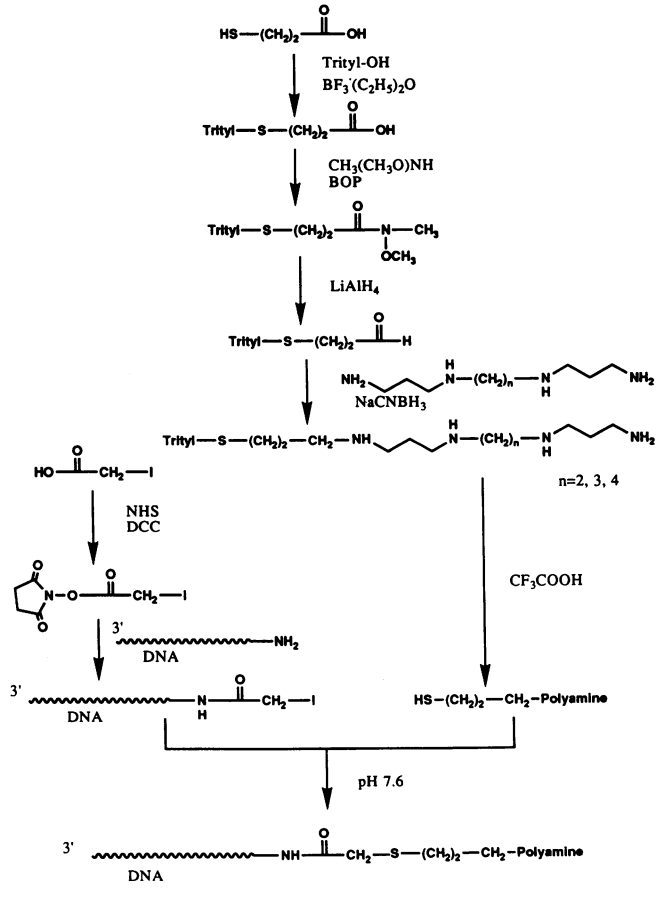

Figure 2. Reaction scheme for preparation of polyamine-oligonucleotide conjugates.

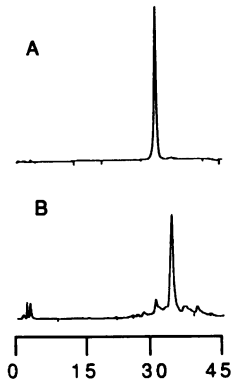

Figure 3. HPLC reverse phase chromatogram of (A) 5' amino-linked Z, and (B) 3,4,3,-Z conjugate.

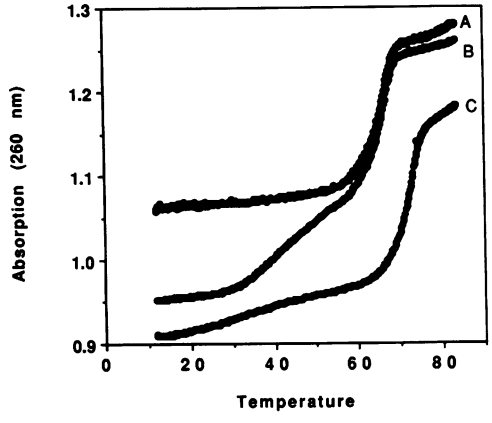

Figure 4. UV melting temperature profiles showing the effect of appended versus free spermine. The absorption change was measured in pH 6.5, 10 mM sodium phosphate buffer, 100 mM sodium chloride. The curve A is XYZ, curve B is XY(3,4,3-Z), and curve C is XYZ with 1 mM spermine in the solution.

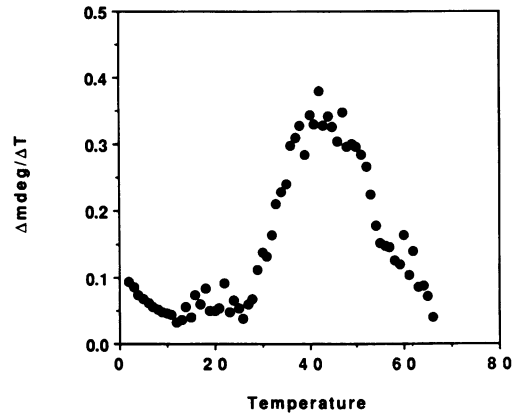

Figure 5. Derivative of ellipticity of XY(3,4,3-Z) CD melting profile versus temperature at 218 nm in 10 mM sodium phosphate buffer (pH 6.5), 100 mM sodium chloride.

Table 1. Melting temperature of duplex and triplex

Third strand	Tm(°C) Duplex	Triplex
No	66	–
Z	67	Not detected
3,2,3-Z	67	38
3,3,3-Z	66	40
3,4,3-Z	67	42
Z(1mM spermine)	73	36
3,4,3-Z(3mM $MgCl_2$)	71	48
Z(3mM $MgCl_2$)	71	46

The transition was measured in 10 mM sodium phosphate buffer, pH 6.5, 100 mM sodium chloride. The endogenous concentration of polycations were indicated.

the thermally-induced release of the third strand, whereas the second transition corresponds to melting of th target duplex into its component single strands. The X, Y and either Z or 3,4,3-Z strands were annealed by incubating at room temperature in 10 mM sodium phosphate, pH 6.5, 100 mM sodium chloride buffer overnight. Inspection of curve A in Figure 4 reveals that the melting profile for the XYZ system, in which the third strand does not have an appended spermine, exhibits no change in absorption between 10 and 50°C. This observation is consistant with the triple helix never being present in the XYZ system. The double-stranded XY had formed and melted at 67°C (Figure 4, curve A). When strand Z was substituted by spermine-conjugated 3,4,3-Z strand, the complex revealed a transition at 42°C, with the XY duplex subsequently melting at 67°C (Figure 4, curve B). When free spermine (1 mM) was added to the XYZ system, a shallow change in absorption was found (Figure 4, curve C), which corresponded to a triple helix melt at about 36°C. Free spermine, however, also increased the melting temperature of the XY duplex to 73°C. These results are summarized in Table 1. Although the concentration of spermine in the 3,4,3-Z strand was only 2 μM, the improvement in triple helix formation was substantially greater than that produced by a 500-fold higher concentration of free spermine. Also the spermine in 3,4,3-Z had no effect on the stability of double-stranded DNA.

It is known that triple helix formation significantly changes the CD spectrum between 200 and 230 nm (28). Thus, melting of the XY(3,4,3-Z) was also examined by CD at 218 nm. Inspection of the data derived from the CD spectra (Figure 5) reveals triple helix melting at 42°C, which is consistant with the result from the UV melting curve.

In the presence of magnesium ion (3 mM), the XYZ and XY(3,4,3-Z) each showed two melting transitions. The difference of triplex thermal stability between 3,4,3-Z and Z is small, 48 and 46°C, respectively (Table 1). The melting temperature of the duplex was increased to 71°C. The added magnesium ion appears to compete with the appended spermine, perhaps interfering with spermine binding to the underlying double helix.

Effect of the different species of polyamine

The four amino groups in spermine are separated by propylene, butylene and propylene groups (Figure 1). It has been demonstrated that the distances between the amino groups of the polyamine are critical to biological function (29). The crystallography studies also showed that every amino group is involved in the major groove binding (18). Therefore, the effect on triple helix formation of different polyamines was evaluated.

Table 2. Melting temperature of duplex and triplex of XY(3,4,3-Z) at different pH

pH	Tm(°C) Duplex	Triplex
6.0	67	43
6.5	67	42
7.0	67	38
7.5	66	37

The transition was measured in 10 mM sodium phosphate buffer, 100 mM sodium chloride.

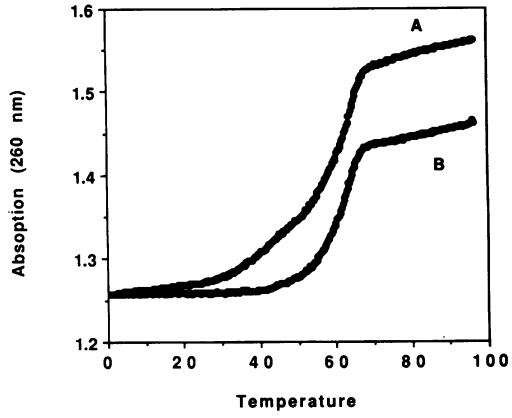

Figure 6. UV melting profiles of (30X)(30Y)(3,4,3-Z) and (30X)(30Y)Z in pH 6.5, 10 mM sodium phosphate buffer, 100 mM sodium chloride. Curve A is (30X)(30Y)(3,4,3-Z) which has two melting points at 43C and 63°C. Curve B is (30X)(30Y)Z which has only a T_m at 63°C.

Besides spermine, N,N′-Bis(3-aminopropyl)-1,3-propanediamine and N,N′-Bis(3-aminopropyl) ethylenediamine were attached to the same 21-mer oligonucleotide. In all three structures, the distances from N1 to N2 and from N3 to N4 are exactly the same, but the gap between N2 and N3 is varied. The samples were prepared and their melting curves were determined in the same way. Although all three appended polyamines effectively promoted triple-strand formation, they promoted smaller increase in T_m as the spacing between N2 and N3 was decreased. Spermine, which is the natural polyamine, gave the greatest enhancement in T_m (Table 1).

Effect of pH on the spermine-conjugated oligonucleotide

The binding of the spermine-oligonucleotide was tested at different pH values (Table 2). The melting temperature of the double-stranded DNA was the same from pH 6.0 to 7.5. For the triple helix, the transition curves were nearly the same (T_m=43 and 42°C), under slightly acidic conditions (pH 6.0 and 6.5, respectively), but there was a marked loss in triple helix thermal stability (T_m=38 and 37°C) in neutral buffer (pH 7.0 and 7.5, respectively). The dramatic change in melting temperature between pH 6.5 and 7.0 may be explained as follows. Each 5-methylated cytosine would be protonated at pH 6.0 and 6.5, resulting in two Hoogsteen hydrogen bonds to stabilize the triple helix structure. Since 5-methylated cytosine would be less protonated at neutral pH, the total amount of hydrogen bonding stabilizing the triple helix would decrease. With the conjugated spermine moiety, however, the third strand can still form a triple helix even at pH 7.5 due to the extra binding energy that results from interaction with the appended polyamine.

Contribution of overhanging bases

A pair of oligonucleotides, 30X and 30Y, having the same sequence as the XY duplex except for seven missing base pairs, was prepared (Figure 1). This region was predicted to be the polyamine binding site. The melting profile was measured under the same conditions to compare the (30X)(30Y)(3,4,3-Z) and XY(3,4,3-Z) systems. The (30X)(30Y)(3,4,3-Z) mixture had melting temperatures for triplex and duplex at 43°C and 63°C, respectively, whereas (30X)(30Y)Z had only one transition at 63°C (Figure 6). That is, triplex thermal stability was essentially the same in the presence and absence of the overhang duplex (Figure 1). This observation reveals that major groove binding of the polyamine may not be responsible for triple helix stabilization by the polyamine-conjugated oligonucleotide. If the polyamine had to lock into the major groove, the triple helix should not form in the absence of the overhanging base pairs. Instead, the polyamine must fold back and interact with the triple-stranded region.

CONCLUSION

Millimolar levels of spermine (and/or divalent magnesium) are typically used for *in vitro* triple helix studies. For the application of synthetic oligonucleotides as antigene inhibitors of transcription in whole cells, it is not possible to modify the intranuclear millieu. In this report, therefore, we investigated the modification of the oligonucleotide so that it would not be dependent on intranuclear polyamine content. A series of polyamines was covalently attached to a homopyrimidine oligonucleotide. We found that appended polyamine eliminated the requirement for free polyamine or magnesium ions in triple helix formation. Indeed, the appended polyamine promoted greater enhancement in the thermal stability of the resulting triple helix, as revealed in the UV and CD melting profiles. Our results suggest the possibility of having other appended polycations which may result in an even greater enhancement of triple helix stabilization. An important concern, however, is the specificity of triple helix formation. Potentially, the polyamine moiety may enhance binding to mismatched, non-target double-stranded DNA. Future studies will address this issue and the possibility that variations in the polyamine structure may favor certain target sequences and, hence, contribute to the specificity.

For the purpose of a therapeutic agent, *in vivo* stability and cellular uptake are important concerns. End-blocked oligonucleotides have been shown to be more nuclease resistant (4, 30). In our approach, the polyamine can be conveniently linked to the 3′, 5′ or both ends of an oligonucleotide to improve its nuclease resistance. With regard to cellular uptake, the positive charges on the polyamine might partially neutralize the negative charges on the oligonulceotides, thereby helping internalization. Furthermore, using the polyamine transport system for drug delivery has been reported (31), and this may be applicable to our polyamine tethered oligonucleotides.

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REFERENCES

1. Chubb, J. M., and Hogan, M. E. (1992) Trends Biotech. **10**, 132–136.
2. Thuong, N. T., and Helene, C. (1993) Angew. Chem. Int. Ed. Engl. **32**, 666–690.
3. Postel, E. H., Flint, S. J., Kessler, D. J., and Hogan, M.E. (1991) Proc. Natl. Acad. Sci. USA **88**, 8227–8231.
4. McShan, W. M., Rossen, R. D., Laughter, A. H., Trial, J., Kessler, D. J., Zendegui, J. G., Hogan, M. E., and Orson, F.M. (1992) J. Biol. Chem. **267**, 5712–5721.
5. Povsic, T. J., and Dervan, P. B. (1989) J. Am. Chem. Soc.**111**, 3059–3061.
6. Koh, J. S., and Dervan, P. B. (1992) J. Am. Chem. Soc. **114**,1470–1478.
7. Ono, A., Ts'o, P. O., and Kan, L.-S. (1992) J. Org. Chem. **57**, 3225–3230.
8. Kiessling, L. L., Griffin, L. C., and Dervan, P. B. (1992) Biochemistry **31**, 2829–2834.
9. Krawczyk, S. H., Milligan, J. F., Wadwani, S., Moulds, C., Froehler, B. C., and Matteucci, M. D. (1992) Proc. Natl. Acd. Sci. USA **89**, 3761–3764.
10. Davison, E.C. and Johnsson, K. (1993) Nucleosides Nucleotides **12**, 237–243.
11. Jetter, M. C. and Hobbs, F. W. (1993) Biochemistry **32**, 3249–3254.
12. Mergny, J. L., Duval-Valentin, G., Nguyen, C. H., Perrouault, L., Faucon, B., Rougee, M., Montenay-Garestier, T., Bisagni, E., and Helene, C. (1992) Science **256**, 1681–1684.
13. Hanvey, J. C., Williams, E. M., and Besterman, J. M. (1991) Antisense Res. Dev. **1**, 307–317.
14. Collier, D. A., Thuong, N. T., and Helene, C. (1991) J. Am. Chem. Soc. **113**, 1457–1458.
15. Giovannangeli, C., Rougee, M., Garestier, T., Thuong, N. T., and Helene, C. (1992) Proc. Natl. Acad. Sci. USA **89**, 8631–8635.
16. Grigoriev, M., Praseuth, D., Guieysse, A. L., Robin, P., Thuong, N. T., Helene, C., and Harel-Bellan, A. (1993) Proc. Natl. Acad. Sci. USA **90**, 3501–3505.
17. Tabor, H. (1962) Biochemistry **1**, 496–501.
18. Jain, S., Zon, G., and Sundaralingam, M. (1989) Biochemistry **28**, 2360–2364.
19. Arnott, S., and Selsing, E. J. (1974) J. Mol. Biol. **88**, 509–521.
20. Macaya, R., Wang, E., Schultze, P., Sklenar, V. and Feigon, J. (1992) J. Mol. Biol. **225**, 755–773.
21. Howard, F. B., Miles, H. T., Liu, K., Frazier, J., Raghunathan, G., and Sasisekharan, V. (1992) Biochemistry **31**, 10671–10677.
22. Ouali, M., Letellier, R., Adnet, F., Liquier, J., Sun, J.-S., Lavery, R. and Taillandier, E. (1993) Biochemistry **32**, 2098–2103.
23. Fehrentz, J.-A. and Castro, B. (1983) Synthesis 676–678.
24. Kido, K., Inoue, H., and Ohtshka, E. (1992) Nucleic Acids Res. **20**, 1339–1344.
25. Schmidt, F. J., Bock, R. M., and Hecht, S. M. (1972) Biochem. Biophys. Res. Commun. **48**, 451–456.
26. Tung, C.-H., Rudolph, M. J., and Stein, S. (1991) Bioconjugate Chem. **2**, 464–465.
27. Plum, G. E., Park, Y.-W., Singleton, S. F., Derevan, P. B. and Breslauer, K. J. (1990) Proc. Natl. Acad. Sci. USA **87**, 9436–9440.
28. Park, Y.-W., and Breslauer, K. J. (1992) Proc. Natl. Acad. Sci. USA **89**, 6653–6657.
29. Jorstad, C. M., Harada, J. J. and Morris, D. R. (1980) J. Bacteriol. **141**, 456–463.
30. Tang, J. Y., Temsamani, J. and Agrawal, S. (1993) Nucleic Acids Res. **21**, 2729–2735.
31. Holley, J. L., Mather, A., Wheelhouse, R. T., Cullis, P. M., Hartley, J. A., Bingham, J. P., and Cohen, G. M. (1992) Cancer Res. **52**, 4190–4195.