Covalent Immobilization of Nucleic Acid Probes onto Reactive Synthetic Polymers

N. FERRATON,[1] TH. DELAIR,[2] A. LAAYOUN,[1] P. CROS,[1] B. MANDRAND[2]

[1] Groupe de Chimie des Acides Nucléiques, bioMérieux, Parc Club du Moulin à Vent, 33, Av. Lévy, 69693 Vénissieux, France

[2] Unité Mixte CNRS-bioMérieux, UMR-103, ENS-Lyon, 46, Allée d'Italie 69364 Lyon Cédex 07, France

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ABSTRACT: Oligodeoxyribonucleotide (ODN)/polymer conjugates were obtained by covalent immobilization of various nucleic acid sequences to three different polymers. Two of them bore active ester groups for the covalent linkage via the formation of amide bonds and the third one featured aldehyde moieties for immobilization via reductive amination. The factors controlling the conjugation reaction were found to be the reactivities of the polymers and their abilities at forming hydrogen bonds with the ODNs to be tethered. It was found, as well, that the observed aggregation of the grafting reaction products was essentially due to hydrogen bonding with the nucleic bases of the tethered oligonucleotides. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **66:** 233–242, 1997

Key words: oligodeoxyribonucleotides; immobilization; functional polymers; aggregation

INTRODUCTION

Linear synthetic functional polymers have proved very useful in various diagnostics applications. For instance, they have been bound to proteins and oligonucleotides to increase the coating efficiency of allergens[1] and nucleic acid probes[2] or to increase the detection signal in various tests.[3,4] For diagnostics purposes, we investigated the binding of nucleic acid probes to water-soluble polymers of various chemical structures.[4–9] Here, we report on a comparative study on the course of the grafting reactions and conformations (in particular, the aggregation issue) of the resulting reaction products, using two activated ester-based copolymers (immobilization via an amide bond formation) and one aldehyde-based copolymer (grafting via the formation of an imine, subsequently reduced to the corresponding alkylamine). The results will be discussed in terms of chemical reactivities of the polymers, of base composition and sequence of the probes to be tethered, and of macromolecular interactions.

Correspondence to: Th. Delair.

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EXPERIMENTAL

Chemicals

Oligodeoxyribonucleotides

Oligonucleotides (Fig. 1) (ODN or oligos or DNA probes) were synthesized at bioMérieux S.A. with a Model 394 Applied Biosystem DNA/RNA synthesizer using cyanoethyl phosphoramidite chemistry on a 1 μmol scale according to the manufacturer's protocols. All the reagents and standard phosphoramidites were from Applied Biosystems (Foster City, CA, USA), except the aminolink II phosphoramidite which was purchased from Clontech Laboratories, Inc. (Palo Alto, CA). The cleavage of synthesized oligodeoxynucleotides from the solid support was carried out automatically on the

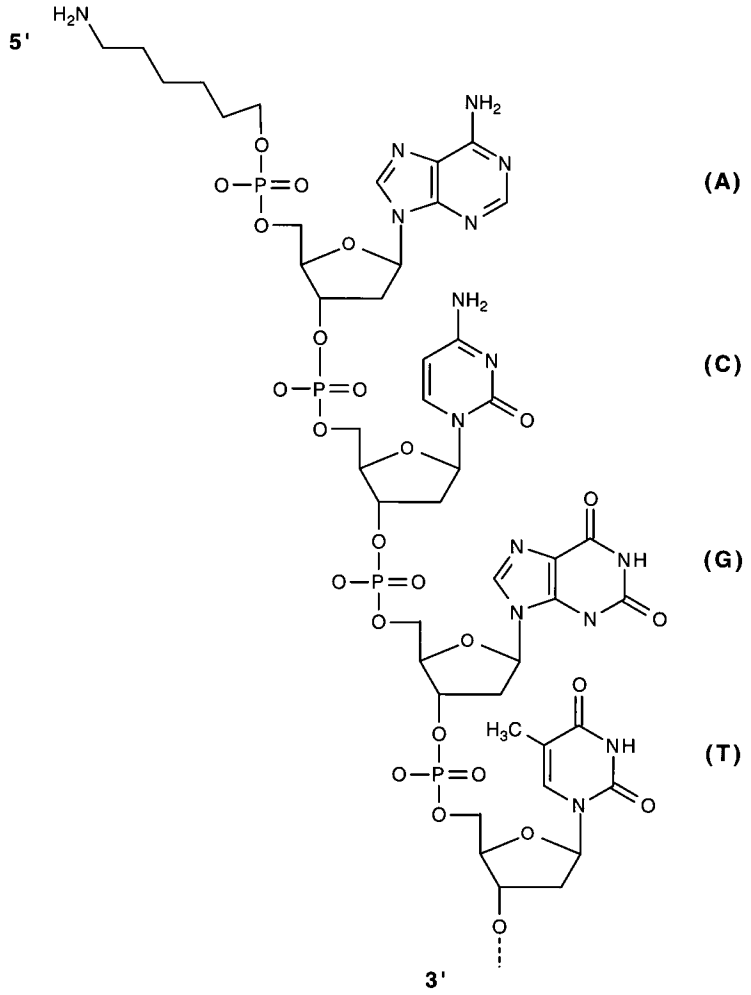

Figure 1 Schematic representation of an oligodeoxyribonucleotide with the four bases A, T, G, C, and the hexamethylene amino spacer.

synthesizer by a 90 mn-long treatment with a 30% ammonium hydroxide solution. The full deprotection was then achieved by incubating, overnight at 55°C, the ammonia solution containing the cleaved oligonucleotide. The oligonucleotides were recovered after precipitation in 3*M* sodium acetate and cold ethanol (−20°C), quantified by measuring UV absorption at 260 nm and stored in solution in water. For the coupling reactions, the above aqueous solution were used as such.

Polymer Samples

The copolymer of maleic anhydride and methylvinyl ether (sample MAMVE-1) was supplied by Polysciences, Inc. The saccharidic copolymer was obtained as described previously.[10] Its composition was 36% hydroxyethyl methacrylate (HEMA) and 64% saccaridic derivative (sample COPO, M_n = 176,000 g/mol). The NVP/NAS copolymer was synthesized according to Ref. 11 as an alternate copolymer (sample NVP/NAS LP-1, M_n ca. 70,000 g/mol).

Coupling of Oligonucleotides to the Reactive Polymers

The grafting procedures were described in previous publications[4,6–9]; nevertheless, a brief description is given below for each type of polymer.

Saccharidic Copolymer (Fig. 2)

Dimethylformamide (DMF) was added to a solution of oligonucleotide dissolved in 20 m*M* borate buffers pH 9.3 or 7.3 and then the polymer in solution in water was added. Coupling was run for 5 days at 50°C under stirring. Then, the imi-

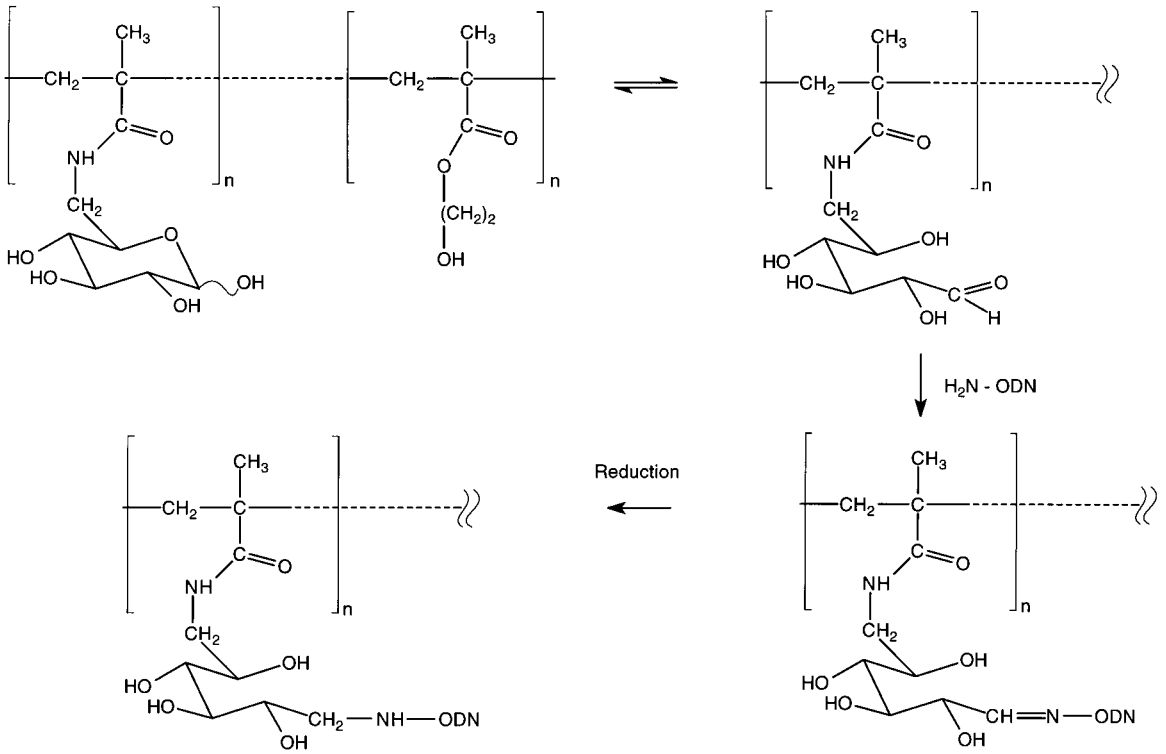

Figure 2 Conjugation reaction between the saccharidic copolymer and an aminated oligonucleotide (ODN).

nine bond reduction was performed by adding aqueous sodium borohydride to the reaction mixture.

NVP/NAS Copolymer (Fig. 3)

DMF was added to a solution of oligonucleotide dissolved in 100 m*M* borate buffers, pH 9.3; then, the polymer in solution in DMF was added. Coupling was run for 2 h at 50°C under stirring.

MAMVE-1 Copolymer (Fig. 4)

Dimethylsulfoxide (DMSO) was added to a solution of oligonucleotide dissolved in 100 m*M* borate buffer, pH 9.3, and 0.5*M* NaCl, and, then, the polymer in solution in DMSO was added. Coupling was run overnight at 37°C under stirring. When experiments were run in triplicate, the reported reaction yields were the mean values of three determinations ± the standard deviation.

Analyses of the Coupling Reactions

The coupling yield was defined as the ratio of the amount of polymer bound ODN vs. the total amount introduced in the reaction mixture. Crude products were purified by size-exclusion chroma-

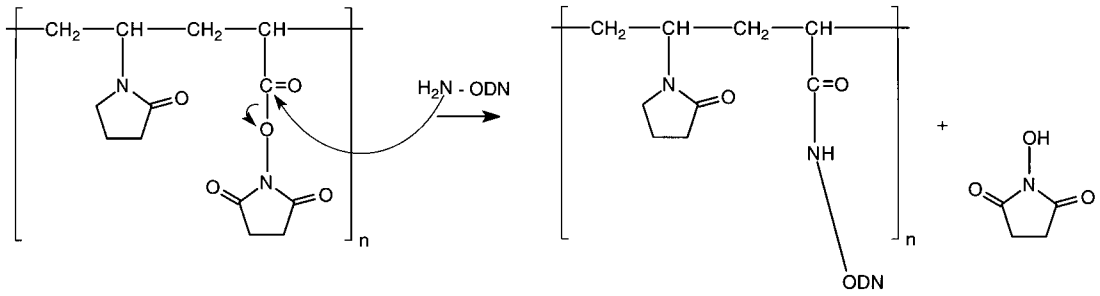

Figure 3 Conjugation reaction between the NVP/NAS copolymer and an aminated oligonucleotide (ODN).

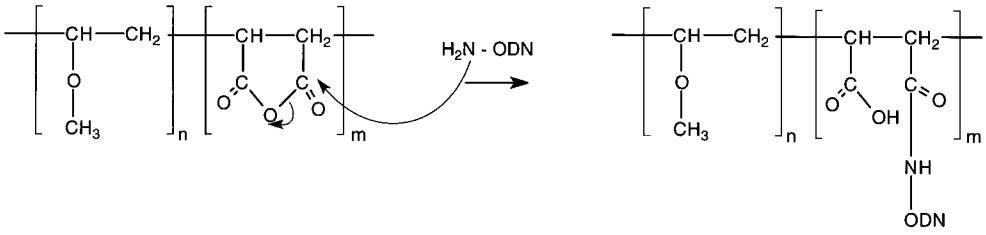

Figure 4 Conjugation reaction between the MAMVE copolymer and an aminated oligonucleotide (ODN).

tography (SEC) using a Waters Ultra-Hydrogel 500 column, a Kontron HPLC 420 pump, and a Kontron 430 UV detector. Purifications were run in a 0.1*M* phosphate buffer, pH 6.8. Detection was achieved by measuring the absorbance at 260 nm corresponding to the ODN. The ratio of the peak area corresponding to the polymer-bound oligonucleotide vs. the sum of the two peaks corresponding to the unbound and the bound oligos (ODN) (i.e., the total amount of probe involved in the reaction) gave the coupling yield, as bound and unbound oligonucleotides have the same specific extinction coefficients.[9]

RESULTS

Factors Controlling the Course of the Grafting Reaction

In the first series of experiments reported in Table I, 12 different oligonucleotides were tested for coupling. Runs 1–4 aimed at evaluating the role of each base independently, since for oligos 2, 3, and 4, six bases of polyT, ODN 1, were substituted, respectively, by G, C, and A. In experiments 5–7, the role of the doublets GA, CG, and CA was studied, and in runs 8–11, the effect of base substitution (C, G, A by T) from sample 8 that bore the three base repeat CGA was assessed. Experiment 12 was designed to determine whether the positions along the ODN sequence of the aminolinker and of the T_6 tail had any effect on the course of the reaction (compare to run 8). Every oligo sample bore a polyT tail so as to minimize eventual nonspecific interactions and to allow oligos to bear enough bases for optimum coupling.

First, as a general trend, the coupling efficiency decreased from the MAMVE copolymer through NVP/NAS, the saccharidic copolymer at pH 7.3, and, finally, the saccharidic copolymer at pH 9.3. The polyT, ODN 1, gave the highest immobilization yields with activated ester-based copolymers. With saccharidic copolymers, oligos bearing the CG doublet were the most efficient at reacting

Table I ODN Base Composition Effect on the Course of the Coupling Reaction with Various Copolymers, When the Indicated Reaction Yields Are the Means of Three Determinations ± Standard Deviation

Entry	ODN Sequence (5′ to 3′)	MAMVE-1 % Yield	NVP/NAS-LP-1 % Yield	COPO – 7.3 % Yield	COPO – 9.3 % Yield
1	T_{18}	71 (±3.5)	69 (±1)	20 (±1)	21 (±3)
2	G_6T_{12}	32 (±3.5)	15 (±10)	47 (±3.5)	36 (±5)
3	C_6T_{12}	67 (±0.5)	51 (±2)	28 (±3.5)	23 (±0.5)
4	A_6T_{12}	57 (±1)	53 (±3)	12 (±1.5)	16 (±1.5)
5	$(GA)_4T_{10}$	63 (±0.5)	50 (±4.5)	17 (±1)	36 (±1)
6	$(CG)_4T_{10}$	68 (±1)	58 (±2.5)	51 (±5)	35 (±3)
7	$(CA)_4T_{10}$	66 (±1.5)	61 (±2)	20 (±2.5)	21 (±1)
8	$(CGA)_4T_6$	61 (±0.5)	46 (±2)	54 (±2)	26 (±1.5)
9	$(CTA)_4T_6$	66	19	23	18
10	$(CGT)_4T_6$	64	49	44	37
11	$(TGA)_4T_6$	60	38	22	34
12	$T_6(AGC)_4$	64 (≤ 1)	49 (±1)	56 (±1.5)	14 (±3)

with the aldehydic moieties of the synthetic macromolecule at both pH's (runs 6, 8, 10). In more detail, at the oligo base composition level, ODN 2, bearing the polyG head, prone to the formation of quadruplex[12] at high salinity, was the one sample that gave the poorest coupling yields for the MAMVE and NVP/NAS polymers as compared to the 12 different sequences investigated in Table I. For the saccharidic copolymer, which requires a low ionic strength for the immobilization reaction to take place,[7,8] ODN 2 gave rather high coupling yields but ODN 4, highly susceptible to self-associations due to the presence of polyA and polyT sections, was poorly tethered onto the synthetic macromolecule.

Investigating the role of base doublets GA, CG, and CA in experiments 5, 6, and 7 for the saccharidic copolymer under both experimental conditions, it turned out that ODN 7, missing the G base, was less efficient in the grafting process. ODN 6 containing CG doublets gave high immobilization yields at both pH's. For the two activated ester-based copolymers, little difference in reactivity was observed for the three investigated base doublets.

From runs 8–12, where the effect, on the course of the reaction, of the substitution of the G, C, and A bases was studied, it turned out that the MAMVE copolymer, with a respective coupling yield of 61, 66, 64, and 60%, was far less sensitive to ODN base composition modifications than were the two others. With the NVP–NAS and saccharidic copolymer at both pH's, ODN 9, missing the G base, always bound to the polymers with lower yields than did ODNs 8, 10, and 11. Finally, changing the position of the aminolinker and the polyT tail from the 5′ to the 3′ position of the oligonucleotide (respectively, runs 8 and 12) had no effect on the course of the reaction except for the saccharidic copolymer at pH 9.3.

To assess to what extent the bases of the DNA probes were involved in the interactions that allowed the chemical immobilization reaction to take place, polyT oligonucleotides were synthesized with varying contents of *N*-methylated T (Fig. 5). Methylated ODNs 14 and 15, later called homogeneous sequences, as well as mixed sequences ODN 17, 18, and 19 were coupled to activated ester-based and saccharidic copolymers. Immobilization yields for these methylated sequences, and for the unmethylated standard homogeneous sequence 13 and mixed sequence 16, are reported in Table II. Digit X in the sequences represent N_3-methylated T. With these samples, the values of the coupling yields for each copolymer was a means to assess the influence, on the course of the reaction, of the level of N-methylation of the starting oligonucleotides. In the homogeneous sequences, an increase in the N-methylated T contents resulted in a drastic drop in coupling yields (runs 13–15). Once again, the MAMVE-1 copolymer was less sensitive to base composition modifications than were the two others. In the mixed series, for the MAMVE sample, the coupling yields were not affected on increasing the X contents in the probes. The effect of methylation in the mixed series was more marked for the saccaridic copolymer than for the NVP/NAS sample.

Factors Controlling the Aggregation of the Conjugates

In Table III are reported the elution times of the polymer/ODN conjugates obtained for ODN 1–12. An elution time inferior to 10 min is typical of a fully aggregated conjugate and is excluded from the SEC column, whereas an elution time of 12 mn or more indicates a fully nonaggregated conjugate.[4–9]

From results in Table III, the polymer chemical nature appeared to be the main factor controlling the conjugate aggregation. For example, conjugates obtained by grafting DNA probes to the NVP/NAS polymer were, in most cases, highly aggregated, whereas those resulting from the grafting at pH 7.3 on the saccharidic copolymer were mainly nonaggregated. A coupling medium composition effect on the aggregation of the conjugates was also observed: At pH 9.3, conjugates obtained with the saccharidic copolymer were more often aggregated than at pH 7.3.

One common feature of ODN 2 (G_6T_{12}) from the data of Tables I and III was that the errors on the coupling yields and conjugate elution times were always higher with this sample than with any other. On chemical modification of the bases, via N-methylation, in experiments reported in Table IV, a marked effect on conjugate aggregation can be observed. A decrease in aggregation occurred with increasing methylation of the polyT sample (runs 13, 14, 15). Dealing with the mixed sequences, the reduction of the level of aggregation was obtained, for MAMVE-1 and NVP/NAS-LP-1 samples, for partially methylated T ODN 18 and fully methylated ODN 19. Contents of N-methylated T inferior to 33% were ineffective at reducing the level of aggregation, as shown by run

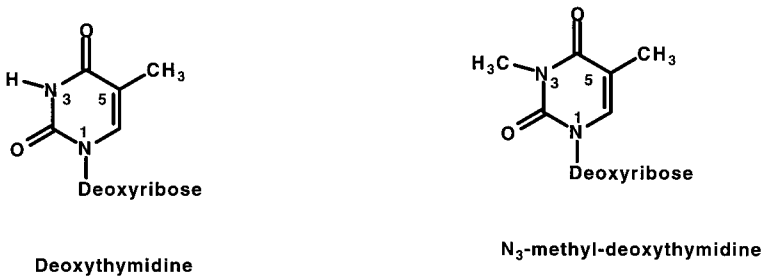

Figure 5 Chemical structure of deoxythymidine and its methylated analog: N_3-methyl-deoxythymidine.

17. For the saccharidic copolymer at pH 9.3, the effect of methylation on the amount of aggregation was fairly drastic as a 22% level of X was enough to reduce the level of aggregation from 51% (run 16) to 18% (run 17).

DISCUSSION

Control of the Course of the Grafting Reaction

The observed polymer reactivity was expected on account of standard organic chemistry knowledge: An anhydride is more reactive than is an *N*-hydroxysuccinimide ester, the least prone to reaction being the aldehyde moiety.

From the data reported in Table I, a careful control of DNA–DNA and DNA–copolymer interactions is required for the immobilization reaction to take place. Any kind of intra- or intersequence associations, as was the case for polyAT (ODN 4) or polyG (ODN 2), resulted in a decrease in coupling yields. So, it is important to note that the initial conformation of the DNA probe to be immobilized plays a predominant role on the course of the grafting reaction. Sequences that can give rise to conformations in which the amino groups of the aminolinker may not be available will result in poor immobilization yields.

The base composition effect was dependent on the ionic strength of the reaction mixture, and, as a consequence, MAMVE-1 and NVP/NAS-LP1 samples have similar behaviors, different from that of the aldehyde-based copolymer, at both pH's. In terms of coupling efficiency, sequences grafted at high ionic strength, in high yields, to the former polymers (for instance, ODNs 1, 3, and 7) were poor sequences for the saccharidic copoly-

Table II ODN N-methylation Effect on the Course of the Immobilization Reaction onto Reactive Polymers

Entry	ODN Sequence (N-methylated T Base %) (5′ to 3′)	MAMVE-1 % Yield	NVP/NAS-LP-1 % Yield	COPO-7.3 % Yield	COPO-9.3 % Yield
13	TTT TTT TTT TTT TTT TTT (0%)	72	70	21	24
14	XTX TXT XTX TXT XTX TXT (50%)	71	4	6	4
15	XXX XXX XXX XXX XXX XXT (95%)	46	7	0	1
16	CGT CGT CGT CGT TTT TTT (0%)	63	49	41	31
17	CGX CGX CGX CGX TTT TTT (22%)	53	67	30	25
18	CGT CGT CGT CGX XXX XXT (33%)	68	66	35	30
19	CGX CGX CGX CGX XXX XXT (50%)	66	18	21	17

X stands for N_3-methylated T.

Table III ODN Base Composition Effect on the Aggregation of Various Oligonucleotide/Copolymer Conjugates as Observed by SEC (Reported Are the Elution Times in mn)

Entry	ODN Sequence (5′ to 3′)	MAMVE-1 mn (±%)	NVP/NAS-LP-1 mn (±%)	COPO-7.3 mn (±%)	COPO-9.3 mn (±%)
1	T_{18}	11.44 (±1.1)	9.48 (±1.2)	12.95 (±1.2)	12.01 (≤ 1)
2	G_6T_{12}	12.24 (±4.9)	9.6 (±3.4)	12.41 (≤ 1)	10.34 (±4.8)
3	C_6T_{12}	11.12 (±2.2)	9.49 (±1)	13.03 (±1.1)	12.07 (±1.7)
4	A_6T_{12}	11.73 (≤ 1)	9.633 (±1.3)	13.3 (≤ 1)	12.35 (±2)
5	$(GA)_4T_{10}$	10.91 (≤ 1)	9.59 (±1.1)	13.46 (≤ 1)	10.21 (±3.8)
6	$(CG)_4T_{10}$	10.51 (±3.4)	9.66 (≤ 1)	13.12 (±1)	10.21 (±3.8)
7	$(CA)_4T_{10}$	10.55 (±3.2)	9.72 (±1.5)	13.35 (≤ 1)	11.87 (±1.3)
8	$(CGA)_4T_6$	10.62 (±3.4)	9.63 (±1.8)	12.86 (≤ 1)	9.97 (±1)
9	$(CTA)_4T_6$	9.84	9.83	13.3	12.1
10	$(CGT)_4T_6$	10.13	9.52	13.2	10.1
11	$(TGA)_4T_6$	9.82	9.46	13.5	10.1
12	$T_6(AGC)_4$	13.38 (±3.5)	9.63 (±1.2)	12.58 (±2.5)	9.93 (±1.5)

mer and vice versa (ODN 2 and 8). Nevertheless, as a general trend, activated ester-based copolymers were less sensitive to ODN base composition than was the saccharidic copolymer, probably because of higher chemical reactivities. Sequences bearing the CG doublets were immobilized with fairly high coupling yields onto the saccharidic copolymer, at both pH's, whereas no base effect really predominated for MAMVE-1 and NVP/NAS-LP1 counterparts, except for the polyG effect.

The next key factor controlling the grafting reaction of DNA probes to synthetic copolymers is the capacity of both macromolecules to interact with one another, as the chemical reaction can only take place if the macromolecules can get close enough to encounter each other. Therefore, some kind of interaction should exist between the molecules, and hydrogen bonding, involving the bases of the oligonucleotides, can be one kind of interactions that can bring the macromolecules together. N_3-methylation of T is a way to suppress this capability of forming hydrogen bonds for polyT sequences as seen from data of runs 13–15 in Table II. These experiments clearly demonstrate that when no hydrogen bonding is possible between

Table IV ODN *N*-methylation Effect on the Aggregation of Oligonucleotide Conjugates with Reactive Polymers

Entry	ODN Sequence (N-methylated T Base %) (5′ to 3′)	MAMVE-1 EP (%)	NVP/NAS-LP-1 EP (%)	COPO-7.3 EP (%)	COPO-9.3 EP (%)
13	TTT TTT TTT TTT TTT TTT (0%)	29	91	0	11
14	XTX TXT XTX TXT XTX TXT (50%)	15	75	0	0
15	XXX XXX XXX XXX XXX XXT (95%)	12	0	0	0
16	CGT CGT CGT CGT TTT TTT (0%)	44	100	0	51
17	CGX CGX CGX CGX TTT TTT (22%)	44	100	0	18
18	CGT CGT CGT CGX XXX XXT (33%)	30	4	0	5
19	CGX CGX CGX CGX XXX XXT (50%)	26	0	0	0

X stands for N_3-methylated T. EP is the amount of the excluded peak.

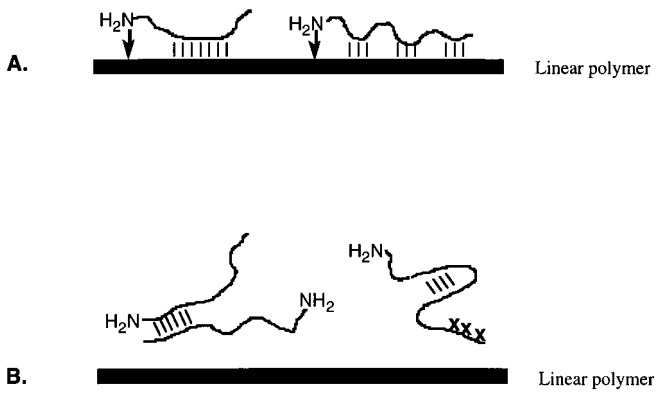

Figure 6 ODN–ODN and ODN–polymer interactions during the conjugation reaction.

the polymer and the ODN molecules (run 15) the grafting reaction cannot proceed. For the highly reactive MAMVE-1 polymer, the immobilization occurred, even at 95% methylation, but the yield was fairly reduced. It is worth noting that when methylation was maintained under 50% of the total base composition (runs 17 and 18), the covalent reaction took place with no drastic alteration of the reaction yields.

From our results, we can suggest a mechanistic approach of the coupling reaction of oligonucleotides to copolymers as depicted in Figure 6. The covalent grafting of an ODN molecule to a polymer molecule occurs via a two-step process: (i) a hydrogen-bond-mediated approach of the two macromolecules and (ii) the chemical reaction between a reactive moiety of the polymer and the amine of the nucleic acid probe (mainly the primary amine group at the end of the hexamethylene spacer) [Fig. 6(A)]. When the approach is not possible because of inter/intrasequence associations, or because the bases are not able to create hydrogen bonds, after methylation, for instance, no coupling can take place [Fig. 6(B)]. From Table II, in the case of the saccharidic copolymer at both pH's and for the MAMVE polymer, there seemed to be a site of methylation on the DNA probe with a greater influence on the course of the grafting reaction. In run 17, where the level of X was of 22% of the total base amount, the immobilization yields were lower than for run 18 for which methylation reached 33%. This result points out that interactions of the polymer with the head of the oligonucleotide, close to the aminated spacer arm, favors the chemical reaction. This is totally in accordance with our proposed mechanism, since if the amino groups cannot easily get close to the reactive moieties of the polymer, the covalent immobilization reaction will be disfavored.

Control of the Aggregation of the Polymer/ODN Conjugates

The formation of aggregates, depicted in SEC HPLC by the presence of excluded peaks, can either be due to (i) chemical crosslinking of reactive polymer chains with the amino groups of the bases or (ii) hydrogen bonding or any other associative process. Chemical crosslinking was suspected to occur, as we demonstrated in various other works that the NVP–NAS copolymer,[4] the MAMVE polymer,[9] and the saccharidic polymers[8] could covalently bind nucleic acid probes devoid of a spacer arm, with an immobilization yield up to 15%. This immobilization reaction is thought to involve the amino groups of the bases. Nevertheless, our results on base methylation, in particular run 18 in Tables II and IV, underline the fact that chemical crosslinking cannot be regarded as the major factor of the aggregation observed on coupling.

Concerning other associative processes capable of inducing aggregation, we have already ruled out inter- or intra-ODN interactions in the case of the NVP/NAS copolymer.[4] Furthermore, from the results in Table III, although some ODN gave more aggregated conjugates than did the others, no apparent base composition effect on the aggregation process could be observed, in opposition to the polymer nature effect, observed in Table IV.

From Table IV, one can notice that, for the saccharidic copolymer and the NVP–NAS copolymer,

the observed aggregation phenomenon was only due to the formation of hydrogen bonds between the bases of the nucleic acid and the macromolecules. This hypothesis is supported by the fact that a high N_3-methylation level of the T base of the probes (95% for the homogeneous sequences, run 15, and 50% for the mixed sequence, run 19) prevented the aggregation process to take place. For the MAMVE-1 polymer sample, since some aggregation remained even at a high N_3-methylation level of the T base of the probes, one may conclude that a part of the aggregation process may be due to self-associative properties of the copolymer itself. This is in accordance with experimental observation in SEC analyses of the MAMVE-1 polymer sample which showed that, despite many experimental conditions tested, some aggregated material still remained in the solution.[13] The NVP–NAS sample features a great tendency to form hydrogen bonds with oligodeoxynucleotides, as we have obtained, with this polymer, only aggregated conjugates, irrespective or the oligo composition (Table III). But this copolymer, unlike the MAMVE sample, has few self-association properties, since a high level of N_3-methylation of the T base of the probes investigated in Table IV allowed complete disappearance of the high molecular mass aggregates. In this context, run 18 is of particular interest since the coupling yield is similar to runs 16 and 17 and the conjugate level of aggregation was reduced from 100% to only 4%.

For the activated ester-based copolymers (NVP–NAS and MAMVE), on the basis of the results obtained in runs 16, 17, and 18 in Table IV, the formation of aggregates during the covalent immobilization of the oligos onto these two polymers occurs by hydrogen bonding via the polyT tails of the ODNs as their N-methylations drastically reduced the amount of aggregation (run 18), whereas methylation of the CGT heads had no effect (run 17) on the aggregation level. For the saccharidic copolymer, since the immobilization is a lengthy process, the reduction of the aggregation is more strictly related to the increase of the contents in methylated base of the ODN and not to the position of the N-methylated bases along the ODN macromolecule. In a sense, for the saccharidic copolymer, the aggregation process and, to a certain extent, the grafting reaction can be viewed as thermodynamically driven, whereas these processes would be more kinetically controlled for the activated ester-based copolymers.

CONCLUSION

The efficiency of the grafting reaction of oligonucleotides to reactive copolymers and the conformation of the resulting conjugates depend primarily on the existence of interactions between the two macromolecules that bring them together, so that the reaction can occur. These interactions are hydrogen bonds since supressing them by methylation of the amino groups borne by the T bases of model probes prevented the reaction to take place in three of the four studied polymer samples and reduced it for the most reactive one. Methylation allowed us to prove, as well, that hydrogen bonding of polymer chains with the nucleic bases of the probes could be responsible of the observed aggregation of the conjugates, but these interactions alone could not explain, for the MAMVE copolymer, why some degree of aggregation remained, even at the highest level of methylation. For this polymer sample, we suspected self-associative properties to be responsible, at least partially, for the aggregation of the conjugates.

The base methylation experiments allowed us to distinguish two different sites of interactions of the nucleic acid probes. For the course of the chemical grafting reaction, N-methylation of nucleic bases close to the 5′ position entailed a reduction in the coupling efficiency, whereas, for the aggregation process, methylation of the bases at the 3′ end proved more effective at reducing the amount of aggregates for the activated ester-based copolymers.

The chemical reactivity of the polymers was a determinant factor as well. The MAMVE-1 copolymer, with highly reactive anhydride groups, always gave high immobilization yields, whereas yields were lower and more dependent on the oligonucleotide composition, with the copolymers based on less reactive aldehyde. For the saccharidic copolymer, conjugate deaggregation was proportional to the N-methylated T content in the mixed sequences. No site effect was observed, as was the case for the activated ester-based copolymers. Finally, the base composition of the probes to be tethered could prove important since any self-association or particular conformation of the oligonucleotide resulted in poor immobilization yields, as the amine was probably not available for the reaction or the formation of hydrogen bonds with the polymer was prevented.

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REFERENCES

1. M. H. Charles, Th. Delair, M. Jaubert, and B. Mandrand, Fr. Pat. 920,325 (March 1992).
2. C. Mabilat, P. Cros, M. N. Erout, M. H. Charles, C. Pichot, and B. Mandrand, Fr. Pat. 9,307,797 (June 1933).
3. B. Mandrand, P. Cros, Th. Delair, M. H. Charles, M. N. Erout, and C. Pichot, Fr. Pat. 9,311,006 (Sept. 1993).
4. M. N. Erout, A. Troesch, P. Cros, and C. Pichot, *Bioconj. Chem.,* **7,** 568 (1996).
5. L. Véron, M. C. de Binicourt, Th. Delair, C. Pichot, and B. Mandrand, *J. Appl. Polym. Sci.,* **60,** 235 (1996).
6. M. N. Erout, A. Elaissari, P. Cros, R. Kurfurst, and C. Pichot, *Int. J. Polym. Anal. Charact.,* **2,** 253 (1996).
7. Th. Delair, B. Badey, A. Domard, C. Pichot, and B. Mandrand, in *Polym. for Adv. Technol.,* **8,** 297 (1997).
8. Th. Delair, B. Badey, M. H. Charles, A. Laayoun, A. Domard, C. Pichot, and B. Mandrand, in *Polym. for Adv. Technol.,* to appear.
9. C. Ladavière, L. Véron, Th. Delair, A. Domard, C. Pichot, and B. Mandrand, *J. Appl. Polym. Sci.,* to appear.
10. B. Badey, P. Boullanger, A. Domard, P. Cros, Th. Delair, and C. Pichot, *Macromol. Chem. Phys.,* **198,** 945 (1997).
11. M. N. Erout, A. Elaissari, C. Pichot, and M. F. Llauro, *Polymer,* **37,** 1157 (1996).
12. A. Il'icheva and V. L. Florent'ev, *Mol. Biol.,* **26,** 367 (1992).
13. C. Ladavière, Th. Delair, A. Domard, C. Pichot, and B. Mandrand, to appear.