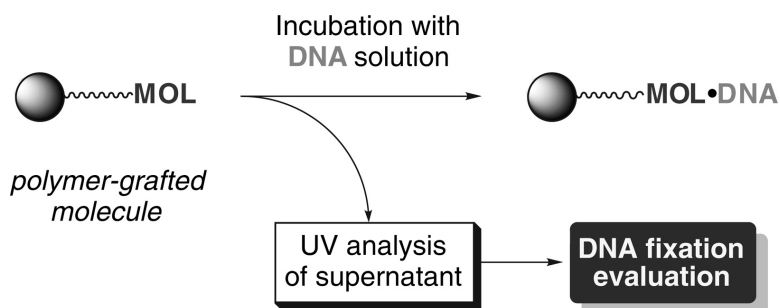Report

Development of a DNA Interaction Test with Small Molecules Still Grafted on Solid Phase

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Reports

Development of a DNA Interaction Test with Small Molecules Still Grafted on Solid Phase

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Gene therapy, as some other approaches for selective delivery of medicines, uses a carrier in order to encapsulate therapeutical DNA and ensure its travel to the desired treatment site.[1] The usual molecular family used to do so is positively charged molecules, such as cationic lipidic or polymeric derivatives. Nevertheless, in the efficiency of such molecules for wrapping and carrying and performing transfection in vitro, cationic DNA vectors suffer from major faults, such as fast clearance in vivo; interactions with biomolecules in the serum, which may lead to strokes; and finally, some cellular toxicity.[2] There is thus still a need for research in order to find new functions and molecules that can advantageously replace cationic vectors in these approaches.

Nowadays, the need for fast synthesis and evaluation techniques to discover new active molecules is one of the organic chemist's major goal. Parallel synthesis in solution, as well as solid-phase approaches, manual or automated, is now currently used to prepare libraries containing several molecules.[3] However, the main drawbacks of such approaches are the speed of analysis and evaluation of libraries for activity.

Usually, molecules synthesized on solid support need to be degrafted from the polymer in order to be tested directly or on another support, such as chips or well plates.[4] There are only a few examples in which the grafted molecule was directly tested or used for its ability to interact with DNA.[5] Other examples include mainly efficient procedures for activity evaluation of peptidic inhibitors toward their target while still grafted on polymers.[6]

During our work on DNA synthetic vectors,[7] we became interested in the modification of their structure, especially the cationic interacting head (Figure 1). However, to test new functional groups for their potential to interact with DNA, we were obligated to conduct complete syntheses before knowing if the new group was even interacting with nucleic acids. We thus needed a relatively fast test in order to evaluate the interacting abilities of new groups or molecules to find leads that could then be incorporated onto vectors and further tested.
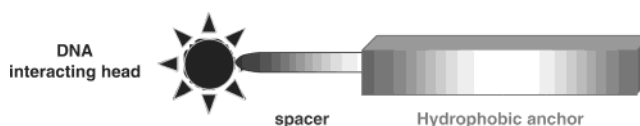
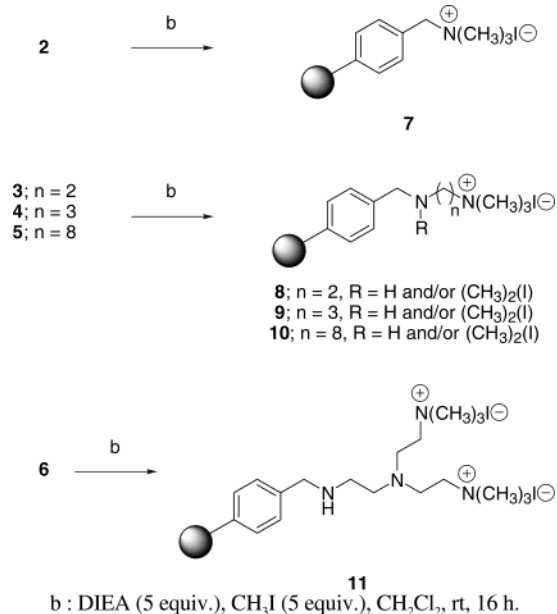**Figure 1.** Schematic representation of synthetic DNA vectors.

Scheme 1. Aminated Polymers Synthesized from Merrifield Resin and Commercially Available

a : $H_2N(CH_2)_nNH_2$ (50 equiv.), DMF, 65°C, 16 h.
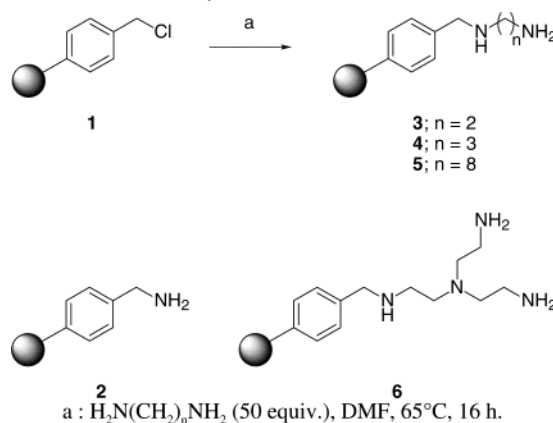
In our continuing efforts to discover new DNA complexing agents for gene therapy and other applications, we present herein our findings during the development of a method to quickly test affinities between molecules and DNA. We chose to test those molecules while still on solid phase as a first evaluation of their ability to interact with DNA. To develop the technique and validate our approach, we first prepared a series of aminated polystyrenes as starting materials for the syntheses of the required functional groups (Scheme 1).

Merrifield resin (**1**, 2.1 mmol g^{-1} Cl) was substituted with alkyldiamines of various lengths by reaction in DMF at 65 °C for 16 h.[8] A diamine excess was used (50 equiv) to prevent reticulation between the sites, to act as the base, and to increase the reaction rate. PS-methyl-(2-aminoethyl)amine (**3**), PS-methyl-(3-aminopropyl)amine (**4**), and PS-methyl-(8-aminooctyl)amine (**8**) were obtained in 93, 91, and 93% coupling efficiencies. The resulting aminated polymers had similar loadings of 1.86, 1.78, and 1.58 mmol g^{-1} NH$_2$, respectively.

The transformations were evaluated by IR analysis of the synthesized polymer. The spectra showed the disappearance of the δ CH$_2$−Cl band (1260 cm^{-1}) from the Merrifield polymer, and observation of new ν N−H (3380−3360 cm^{-1}), δ N−H (1670 cm^{-1}), and ν C−N (1350 cm^{-1}) bands, accounting for NH$_2$/NH groups introduced. Other substitution patterns were selected on the basis of commercially available aminated polymers, such as PS-methylamine (**2**, 1.1 mmol g^{-1} NH$_2$) and PS-methyl-[2-{bis(2-aminoethyl)amino}ethyl]-

Scheme 2. Quaternarization of the Aminated Polymers

b : DIEA (5 equiv.), CH$_3$I (5 equiv.), CH$_2$Cl$_2$, rt, 16 h.

Figure 2. DNA affinity test on ammonium polymers **7−11**: DNA uptake (μg) as a function of time (h).

amine (**6**, 1.17 mmol g^{-1} tris(aminoethyl)amine). Furthermore, all polymers gave a positive result (orange-red coloration) to the qualitative 2,4,6-trinitrobenzenesulfonic acid (TNBS) test,[9] indicative of free primary amine groups on the resin.
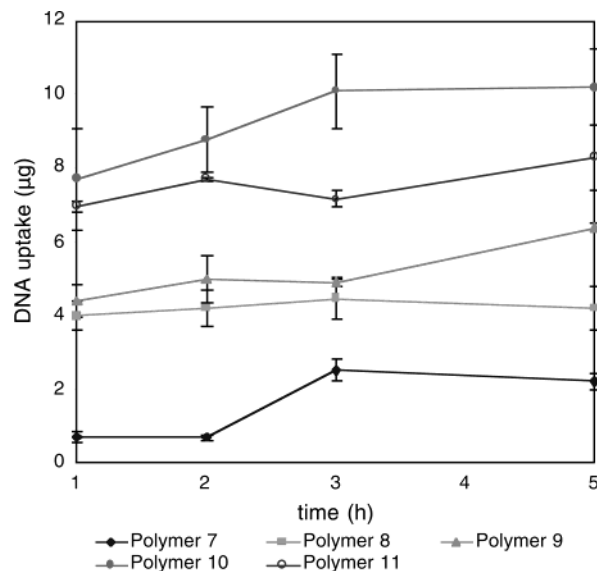
To obtain positively charged polymers, the resins were transformed into their quaternary ammonium salts, known for their ability to interact with DNA (Scheme 2).[1]

Reaction of aminated polystyrenes **2−6** with 5 equiv of methyl iodide for each NH$_2$ group in the presence of Hünig's base gave access to quaternarized ammonium iodide polymers **7−11**, respectively. Coupling efficiencies obtained were 98% (**7**), 105% (**8**), 147% (**9**), 163% (**10**), and 72% (**11**), on the basis of NH$_2$ substitution. Loadings reached the same range for all polymers, between 0.9 and 1.2 mmol g^{-1} N(CH$_3$)$_3$I.

The IR analysis revealed a new δ C−H band (1490 cm^{-1}) due to the methyls incorporated. Modifications of the absorptions of δ N−H (1660 cm^{-1}) and ν C−N (1370 cm^{-1}) bands were observed, as well. Since the ammonium polymers are somehow hygroscopic in nature and still contained some free NH, no clear indication was available on the nitrogen−hydrogen vibration due to a strong absorption at 3420 cm^{-1} on all IR spectrum. Treatment of the polymers with TNBS gave no or little coloration, thus indicating the substitution of primary amine functions.

With these ammonium polymers in hand, we then conducted DNA affinity tests to validate the method parameters and to study the influence of the nature of the spacer between the polymeric structure and the molecule tested. Each of the polymers (20 mg) was mixed together with a DNA solution of known concentration (20 μg/mL)[10] and incubated on an orbital shaker for given time. The solutions were recovered after centrifugation and analyzed at 260 nm to determine the remaining concentration, each incubation for a given polymer being done in triplicate (Figure 2).

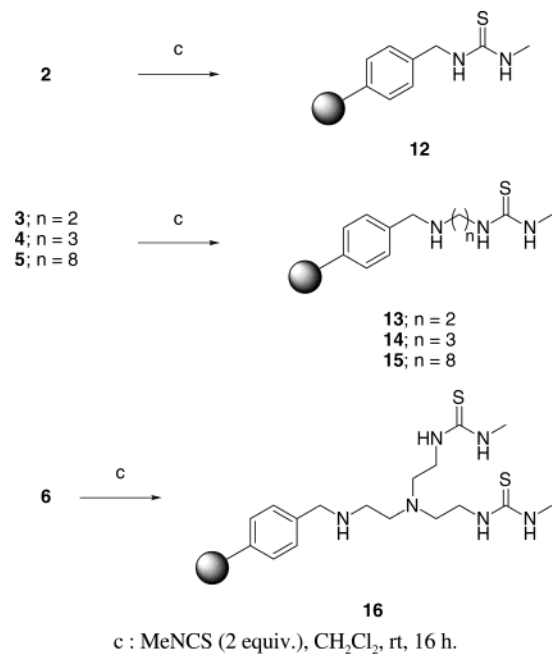From those curves, DNA uptake seems to reach its maximum between 3 and 5 h.[11] The shape of DNA uptake curves in relation to the nature of the polymer give some insight into the capacity of the resin to interact efficiently with this nucleic acid. When the ammonium function was near the polystyrene structure, as in methylated-PS (**7**), withdrawal of DNA from the solution was poor, reaching a value around 2.2−2.5 μg after 3−5 h. Gradually increasing the length of the alkyl spacer from ethyl to propyl and octyl gave much better DNA uptake. For resins **8** and **9**, with ethyl and propyl spacers, the absorptions were of 4.2−4.5 and 4.9−6.4 μg of DNA in 3−5 hours, respectively. A gradual increase of the efficiency to interact with DNA thus seems to be observed and proportional to the spacer length.

This behavior is even clearer when the spacer is octyl, as for polymer **10**, the increase being more regular between 1 and 3 h. The maximum value reached after the 5-h incubation was stable around 10 μg. With polymer **11**, substituted by a structure ending with two antennae bearing the ammonium functions, the results were found to be intermediate. The maximum uptake attained was of 7.2−8.3 μg of DNA for the 3−5 h of soaking.

These results seem to indicate that a better interaction between the ammonium and DNA can be generated by progressively putting the interacting function away from the polymeric structure. Furthermore, they show that the procedure could be used to directly test the affinity toward DNA of supported groups on polymeric support, at least for the ammonium ones.

We next decided to put to the test another functional group that can advantageously replace the problematic cationic part of ionic vectors of DNA. We selected for this purpose the neutral thiourea group, which is known to efficiently complex inorganic anions and has the highest affinity for phosphate ions.[12] The results obtained during a previous study suggested than lipidic polythioureas can complex DNA and may be used as vectors for gene therapy and other applications.[13]

Thus, aminated polystyrenes **2−6** were treated with methyl isothiocyanate (2 equiv) in methylene chloride at room temperature overnight (Scheme 3). Polymers capped
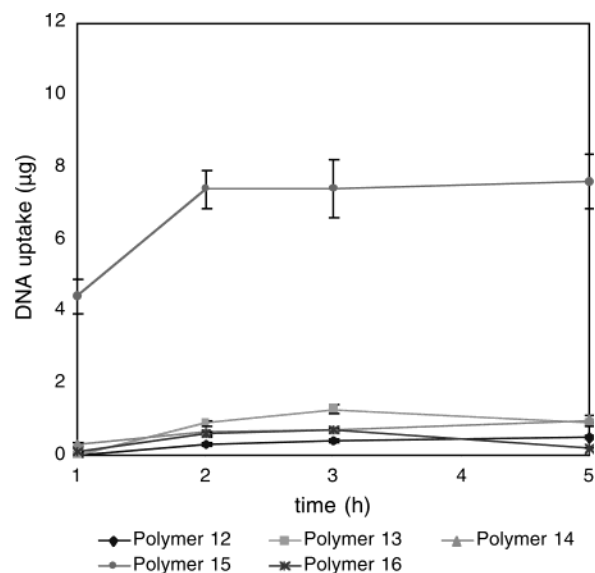
Scheme 3. Synthesis of *N*-Methylthiourea Polymers

c : MeNCS (2 equiv.), CH$_2$Cl$_2$, rt, 16 h.

Figure 3. DNA affinity test on thiourea polymers **12−16**: DNA uptake (μg) as a function of time (h).

with a *N*-methylthiourea function were obtained with the same spacer distribution as for the ammonium ones. The efficiencies reached here were 88% (**12**), 112% (**13**), 115% (**14**), 110% (**15**), and 79% (**16**), on the basis of NH$_2$ reaction. Substitutions of these resins were between 1.6 and 1.8 mmol g^{-1} HN(C=S)NHMe, except for 12, which had a 0.9 substitution value.

IR analyses of the newly synthesized polymers were consistent with at least the transformation of primary amines into thioureas. A new absorption band was observed around 1530 cm^{-1}, attributed to ν C=S vibration. There was also a modification of the ν N−H bands, which appeared centered at 3300 cm^{-1}, and more complex ν C−N bands around 1250 cm^{-1}. The polymers failed to give any, or only a faint, coloration in the TNBS test, one again showing substitution of primary amines.

The methylthiourea polymers were then tested for their affinity toward DNA according to our method (Figure 3). The shapes of the plotted curves were similar to those of the ammonium polymers; however, the uptake levels were quite different. If the methylthiourea was directly grafted onto the polymeric structure, as in **12**, or separated by an ethyl or propyl spacer, as in polymers **13** and **14**, DNA absorption reached only 0.5−1.0 μg after 5 h. Even for the branched resin **16**, DNA capture merely attained 0.2 μg; however, the structure, including the methylthiourea and an octyl spacer on resin **15**, efficiently fixed 7.6 μg of the nucleic acid.

Compared to the ammonium polymers, the fact that a relatively short spacer length (**12−14**), and the spatial disposition of **16**, does not ensure a fixation at all is interesting. This may be caused by the nature of the polymers themselves, preventing the contact between DNA and the thiourea group. The exact nature of the strong influence of spacer length and distribution remains unclear. However, we think that the hydrophobicity of the thiourea resins, when compared to the ammonium ones, can be ruled out, since

polymer **15**, incorporating an octyl spacer, is the only one giving significant results.

The lack of fixation on resins **12−14** and **16** can be the result of a lower availability of the thiourea groups, either by a folding of the arms due to internal interactions or simply by their short distance from the network.

With thioureas, the observations gathered herein can also be an indication of an important steric requirement for bonding, the fixation itself needing a much more intimate interaction than we first believed.[14] For thioureas, bidentate hydrogen bonding should be implicated, which may require a closer contact with the phosphate ions of the DNA structure than the simpler ionic interaction.[15]

We have presented herein the development of a fast affinity test for DNA that can be used with molecules still grafted on a polymeric support. The use of ammonium salts gave us the opportunity to find the best parameters, as well as to study the spacer length influence. Thioureas were tested for their capacity to complex DNA, and found to do so only if steric requirements and a distance from the polymer were met. This test can thus be used to evaluate DNA affinity as well as as a probe for the interaction mechanism. The results obtained during this study confirm than thioureas can interact with DNA on solid phase, and then the test could be used to search for chemical functions for gene therapy and other applications. Further studies of thiourea substitution influence on affinity will be reported in due course.

Supporting Information Available. Experimental procedures and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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