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Hybridization properties of immobilized nucleic acids

T.R.Gingeras*, D.Y.Kwoh and G.R.Davis

SISKA Diagnostics, Inc. and The Salk Institute Biotechnology/Industrial Associates, Inc., PO Box 85200, La Jolla, CA 92138, USA

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ABSTRACT

The 5'-end attachment of oligonucleotides to dextran supports facilitates the study of the hybridization properties of an immobilized oligonucleotide system. The hybridization properties which were studied include: hybridization capacity and kinetics, hybridization-complex stability, and reagents influencing hybridization efficiency. Results of these experiments reveal that the hybridization efficiencies of support-bound oligonucleotides were 75-80% and 40-50% for single-stranded oligonucleotide targets and long double-stranded targets, respectively. These hybridization efficiencies are dependent upon prehybridizing the support-bound oligonucleotides with dextran sulfate. In addition, comparisons of the relative hybridization efficiencies of the support-bound oligonucleotide and nitrocellulose-based systems have been made which indicate a retention of 13-28% of target sequences on the filters and a detection efficiency of 8-20%.

INTRODUCTION

The nucleic acid hybridization protocol most familiar to molecular biologists involves the detection by radioactively labeled probes of target nucleic acids which have been immobilized on nitrocellulose (NC) or nylon filters. This protocol is derived from the work of Nygaard and Hall (2,3), who were the first to immobilize DNA on nitrocellulose paper, and Denhardt (4) and Gillespie and Spiegelman (5), who detected such fixed nucleic acids with radioactive probes. During this same period, Gilham (6,7) described a chemistry of immobilization in which oligonucleotide-length DNA was covalently attached to cellulose supports primarily through the 5' terminal phosphate groups. This approach was a departure from the procedure described by Nygaard and Hall, and later by Southern (8), because the attachment to the solid support occurred specifically at the 5' end of a short DNA molecule by a covalent linkage, rather than by random attach-

ment along the length of a long DNA molecule through a presumed non-covalent interaction. However, the utility of such oligonucleotide-linked supports was slower in finding application. Eventually, covalently linked oligonucleotide supports were employed to selectively isolate polyA-containing mRNA (9), HeLa cell nuclear RNA (10), and specific tRNA's (11).

The hybridization properties of oligonucleotides have been characterized by a number of techniques (12-15) and have been shown to be exquisitely sensitive in detecting single base differences in hybridization complexes (16,17). The unique hybridization properties of oligonucleotides and the rapid advances in the technology of oligonucleotide synthesis and purification have prompted us to investigate the use of oligonucleotides covalently end-attached to supports as a tool in the detection and diagnosis of pathogenic agents or genetically aberrant conditions.

The accompanying paper (1) describes conditions which can be employed for the 5'-end specific attachment of oligonucleotides to a variety of solid supports, as well as presents the coupling efficiencies and non-specific binding properties of these supports. In this report, we describe one such support-bound oligonucleotide hybridization system and some of its hybridization characteristics.

MATERIALS AND METHODS

Oligonucleotides and Plasmid DNA Preparations:

Oligonucleotides attached to supports or used in solution or filter-based hybridizations were prepared using phosphoramidite chemistry in an Applied Biosystems synthesizer (Model 380A). Purification and characterization of the oligonucleotides are described in the accompanying paper (1). Complementary oligonucleotides 85-134 (5'-TGCTGCTATGCCTCATCTTCTTGTTGGTT-3') and 85-147 (5'-AACCAACAAGAAGATGAGGCATAGCAGCA-3') are each 29 nucleotides in length, while oligonucleotide 85-133 (5'-TGGCTCAGTTTACTAGTGCCATT TGTTCAG-3') is 30 nucleotides long. Plasmid pTB061B was constructed by inserting a 0.9 kb EcoRI fragment containing sequences complementary to each of these oligonucleotides in pBR322.

Large-scale plasmid purifications were performed by an alka-

line lysis method (18). Plasmids pTB061B and pBR322 were used as target nucleic acids and linearized by cleaving at ClaI and BamHI sites, respectively, before any hybridization experiment. Radioactive labeling of plasmids pTB061B and pBR322 was accomplished by filling in the recessed 3' ends with the Klenow subfragment of E. coli polymerase in the presence of one ^{32}P-labeled dNTP and three unlabeled dNTP's (19). For Southern hybridizations, "nick translation" of plasmid DNA's was accomplished using the protocol outlined by Maniatis (19). Denaturation of double-stranded, linearized plasmids was accomplished by heating the DNA in 10 mM Tris, pH 7.4, 1 mM EDTA (TE) at 100°C for four minutes. The extent of denaturation was checked by agarose gel (0.8%) electrophoresis. Oligonucleotides were end-labeled with ^{32}P using T4 polynucleotide kinase (19).

Hybridization Supports and Protocols:

Sephacryl 500 dextran supports (Pharmacia) were used for all the experiments described in this study. Although several coupling strategies were investigated to achieve 5'-end specific attachment of oligonucleotides to these supports, the preferred mode of attachment is the coupling of Sephacryl carboxyl supports to the 5'-end aminohexylphosphoramidate-derivatized oligonucleotide through a water-soluble carbodiimide-mediated attachment (1). Coupling times were one hour for all supports used. Support-bound oligonucleotides were stored at 4°C in TE. Unless otherwise specified, 50 mg (wet weight) of supports were aliquoted into Eppendorf tubes and used for each hybridization experiment.

Optimal hybridization conditions include a prehybridization period of 30 minutes at 37°C in 250 µl of solution containing 5x SSPE (0.75 M NaCl, 50 mM NaH_2PO_4, pH 7.4, and 5 mM EDTA), 0.1% SDS, 10% dextran sulfate, and 1 mg/ml of sonicated salmon sperm DNA. Hybridization of the target nucleic acid [50 fmoles (~1 x 10^{-9} molecules), unless otherwise specified] occurs at 37°C in 250 µl of the same solution without salmon sperm DNA for one hour. After the hybridization, the supports were transferred to a dispo-column (QUIK-SEP) and collected by centrifugation, the hybridization solution was removed, and the supports were washed three times at room temperature with one ml each of 2x SSC (0.30

M NaCl, 0.03 M Na citrate, pH 7.0). The hybridization experiments conducted to study the effects of various agents on the efficiency and kinetics of capture are described in the individual figure legends.

Comparison of Support- and Filter-Based Hybridization Systems:

In the support-based hybridization system, the target nucleic acid is in solution, while the probe DNA is end-attached to the surface of the support. For conventional filter-based hybridization systems, the target nucleic acid is immobilized on the surface of the filter, and detection occurs by using a labeled nucleic acid probe which is contained in solution. To determine the overall efficiency of detection for the filter-based hybridization system, it is first necessary to measure the amount of the target nucleic acid that remains immobilized on the filter after the hybridization protocol is complete (the retention efficiency). With this measurement, it is then possible to calculate the hybridization efficiency by measuring the labeled probe which hybridizes to the remaining immobilized target nucleic acid. The hybridization efficiency is calculated as indicated in Table 2.

The retention efficiency of nitrocellulose filters for immobilized target DNA was determined by using linearized ^{32}P-end-labeled pTB016B. The labeled plasmid DNA was denatured with 0.2 N NaOH at 65°C for 30 minutes, neutralized by making the DNA solution 1 M NH_4OAc, and then filtered through BA85 nitrocellulose (Schleicher and Schuell) at dilutions of 10 and 1 ng using a slot-blot apparatus (Schleicher and Schuell). The filters were air dried and baked under vacuum at 80°C for two hours before being counted. The retention efficiency of the immobilized, labeled plasmid was calculated by counting the filters immediately after the samples were applied and after they were subjected to mock hybridization procedures. There was no significant difference between the counts applied to the filter and the counts retained after baking the filter. This mock hybridization procedure entailed measuring the retention of the counts on the filter after prehybridization for 30 minutes, after hybridization for 1 or 24 hours (with no probe added), after each of three room-temperature washes, and after one 55°C wash. The conditions and solutions used for these steps are described below. The counts

remaining on the filter after the last wash procedure were divided by the counts observed after the filters were baked to determine the overall retention efficiency.

Measurements of hybridization efficiency of immobilized target DNA's were conducted using standard filter-based hybridization procedures (19). One and ten nanograms of pTB061B and lambda DNA were immobilized on nitrocellulose filters by using a slot-blot apparatus, followed by heating the filters at 80°C under vacuum. These target nucleic acids were hybridized with denatured, kinased oligonucleotide 85-134. Filters were prehybridized in 5x SSPE, 1% SDS, and 1x Denhardt's (1% Ficoll, polyvinyl pyrrolidone, and BSA) at 55°C for 30 minutes. Hybridizations were conducted under the same conditions using probes at 1 x 10^6 cpm per ml of hybridization solution for 1, 4, and 24 hours. The filters used with labeled oligonucleotide probe were washed three times in 1x SSPE and 1% SDS (3 minutes per wash) at room temperature and once in the same solution at 55°C for 1 minute. Each slot containing immobilized target DNA was cut out and counted in 5 ml of Ecolite enhancer (Westchem).

RESULTS

The Effect of Prehybridization Treatment on the Support-Based Oligonucleotide Hybridizations:

A prehybridization treatment of nitrocellulose filters containing an immobilized target is a commonly used procedure (19). The principal advantage of such pretreatment is seen in the lowering of the non-specific binding of the labeled probe to the support surface. Because the level of non-specific background is one critical criterion which determines the sensitivity of any assay system, the effect of prehybridization on the support-bound oligonucleotide hybridization system was studied. Complementary and non-complementary, labeled targets of both oligonucleotide and plasmid lengths were hybridized to 85-134 supports which had and had not undergone a prehybridization treatment. Table 1 indicates that a prehybridization step had little or no effect on the non-specific background levels, irrespective of the length of the non-complementary target DNA. However, measurements of the efficiency of hybridization indicated that over a 100-fold range

TABLE 1

EFFECT OF PREHYBRIDIZATION
ON HYBRIDIZATION EFFICIENCY[1]

Targets[2]	fmoles	No Pre-hybridization (% Capture)	Pre-hybridization (% Capture)
Complementary	5	36.5	74.9
Oligonucleotide	50	47.2	79.0
	500	52.5	81.9
Non-complementary	5	0.7	0.8
Oligonucleotide	50	0.5	0.8
	500	0.2	0.6
Complementary,	5	27.4	49.0
Double-stranded,	50	29.8	48.2
Long Target	500	27.2	42.8
Non-complementary,	5	0.7	1.3
Double-stranded,	50	0.8	1.0
Long Target	500	1.7	2.3

1. Hybridization protocols for each of the targets are described in Materials and Methods.
2. Complementary and non-complementary oligonucleotides are 30 nucleotides in length, as is oligonucleotide 85-134 immobilized on the surface of the dextran support. The double-stranded, long, complementary targets are linearized plasmids of 5.1 kb in length, cleaved approximately 190 bases from the position of complementarity. The non-complementing target is 4.3 kb in length, with the complementary region not present.

of complementary target DNA concentrations, the hybridization efficiency is consistently higher with supports that have been prehybridized. Thus, prehybridization appears to be required to achieve optimal capture efficiency by the support-bound oligonucleotides.

Capacity of Support-Based Oligonucleotides to Capture Target Nucleic Acids:

Increasing Target Nucleic Acid Concentrations: The capacity of support-based oligonucleotides to hybridize target nucleic acids [oligonucleotide-length (85-147) and denatured, long, double-stranded DNA (pTB061B)] was studied in a series of experiments in which a constant amount of supports (50 mg) was used to hybridize increasing quantities of target DNA. With quantities

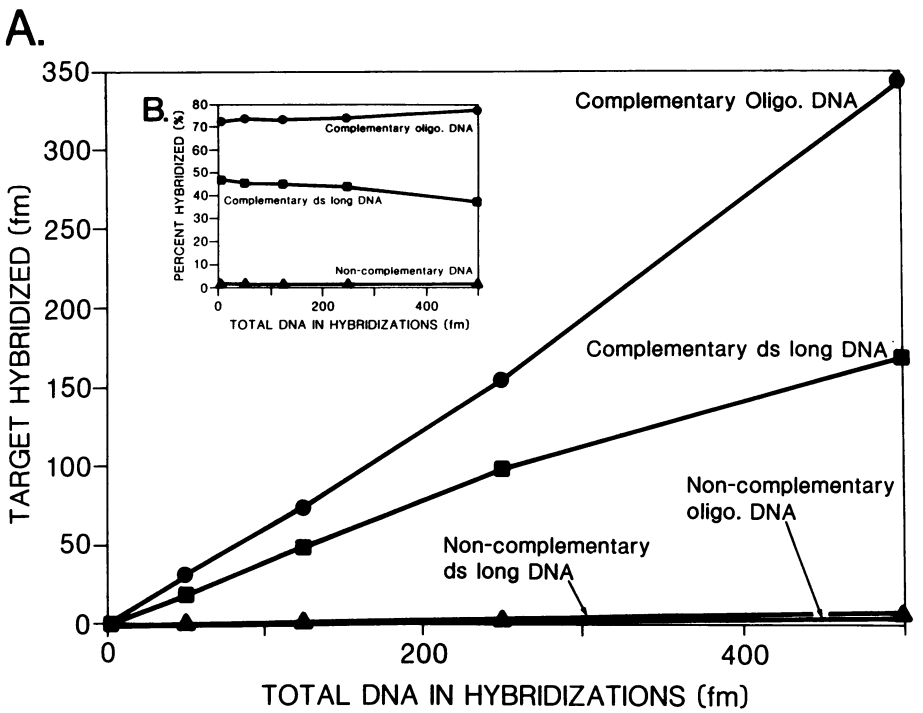

Figure 1. Hybridization of Increasing Quantities of Target DNA. (A) Increasing amounts of complementary oligonucleotide (85-147) and denatured plasmid (pTB061B) were hybridized in each experiment to 50 mg of support-bound oligonucleotides under the conditions described in Materials and Methods. As controls, non-complementary oligonucleotide (85-133) and plasmid (pBR322) were used to measure non-specific adsorption to the support. All target DNA's were labeled with ^{32}P, and the radioactivity associated with the supports was used to determine the quantities of target DNA captured. (B) The quantities of target DNA hybridized to the support-bound oligonucleotides are plotted in terms of percent hybridization. These graphs of hybridization efficiencies demonstrate a constant percent of capture for both long and short target DNA's, irrespective of the target concentration, within the range of target DNA concentration employed.

of target increasing from 5 to 500 fmoles of ^{32}P-labeled DNA, the support-bound oligonucleotides hybridized a constant proportion of the target DNA over this 100-fold range of target (Figure 1A). The efficiency of hybridization levels off at 70-80% for oligonucleotide 85-147 and 40-50% for plasmid pTB061B (Figure 1B). Interestingly, by using a constant amount of support-bound oligonucleotides, the hybridization efficiency does not vary with

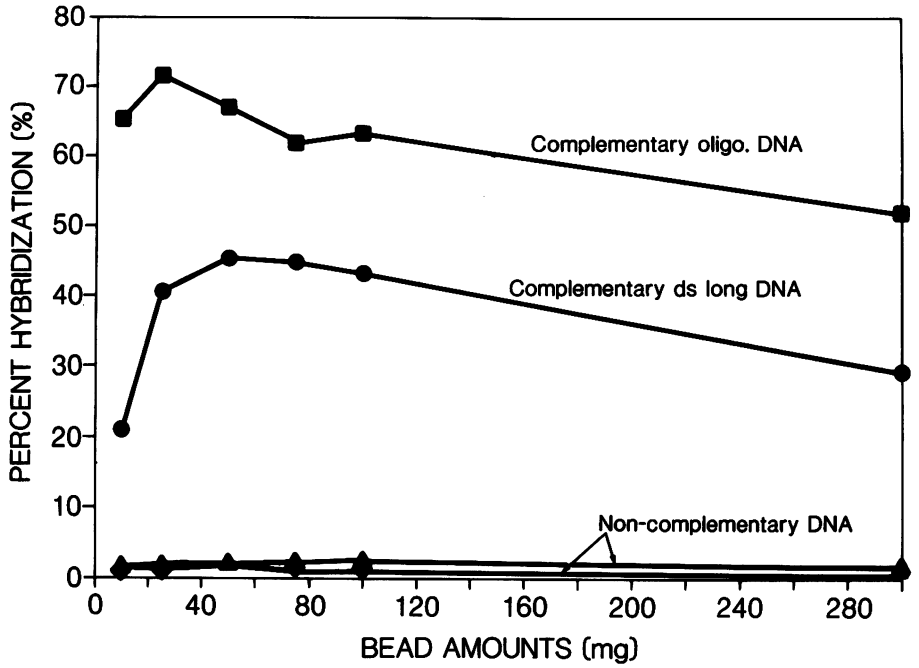

Figure 2. Hybridization of a Fixed Concentration of Target DNA With Increasing Quantities of Supports. Fifty fmoles of the same complementary and non-complementary target DNA's described in Figure 1 were used in hybridization reactions involving increasing quantities (10-300 mg) of support-bound oligonucleotides.

increasing concentrations of target DNA, and only a fixed percentage of the available target is ever hybridized. This result indicates that the capacity of the fixed amount of immobilized oligonucleotides was in excess of target amounts in the 5-500 fmoles range and suggests that only a percentage of the target DNA is capturable. The signal-to-noise ratios in these experiments range from 20-200:1, as measured by retention of ^{32}P-labeled, non-homologous oligonucleotide (85-133) or plasmid (pBR322) targets.

Increasing Support-Bound Oligonucleotide Concentrations: To determine whether it was possible to hybridize all of the ^{32}P-labeled target DNA from solution, the quantity of immobilized oligonucleotide support was increased (from 5 to 300 mg), while the concentration of target DNA was kept constant at 50 fmoles/

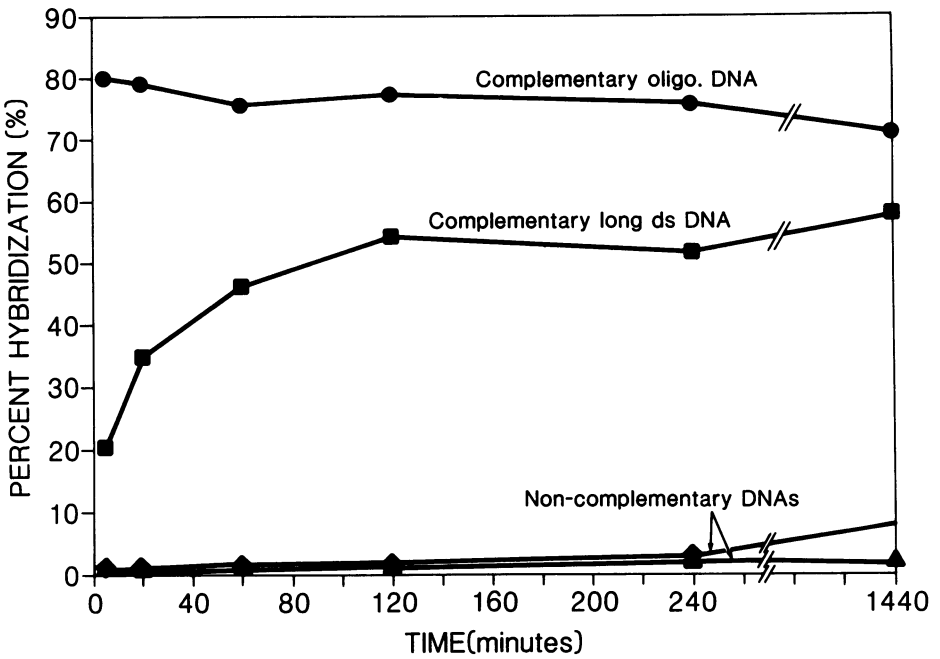

Figure 3. Kinetics of Support-Bound Oligonucleotide Hybridizations. Using 50 mg of support-bound oligonucleotides and 50 fmoles of target DNA's for each reaction, the hybridization efficiencies were measured as a function of time of hybridization. Hybridization times from 5 to 1440 minutes (24 hours) were used.

250 µl of hybridization solution. Figure 2 shows that between 10 mg to 300 mg of support, a relatively constant percent of ^{32}P-labeled target DNA (long and short) is captured, and the levels of the hybridization efficiencies are consistent with those observed in Figure 1B. However, there is a small decrease in the efficiencies of capture (from 65% to 57% for 85-147 and from 55% to 45% for pTB061B) at higher support-bound oligonucleotide concentrations. These results again suggest that the availability of target DNA is the limiting factor in the complete capture of target from solution. Optimal hybridization efficiencies of this system are obtained when 25-50 mg of support per 250 µl of hybridization solution are used for capture. The levels of non-specific background for these experiments are low and show signal-to-noise ratios similar to those observed in Figure 3.

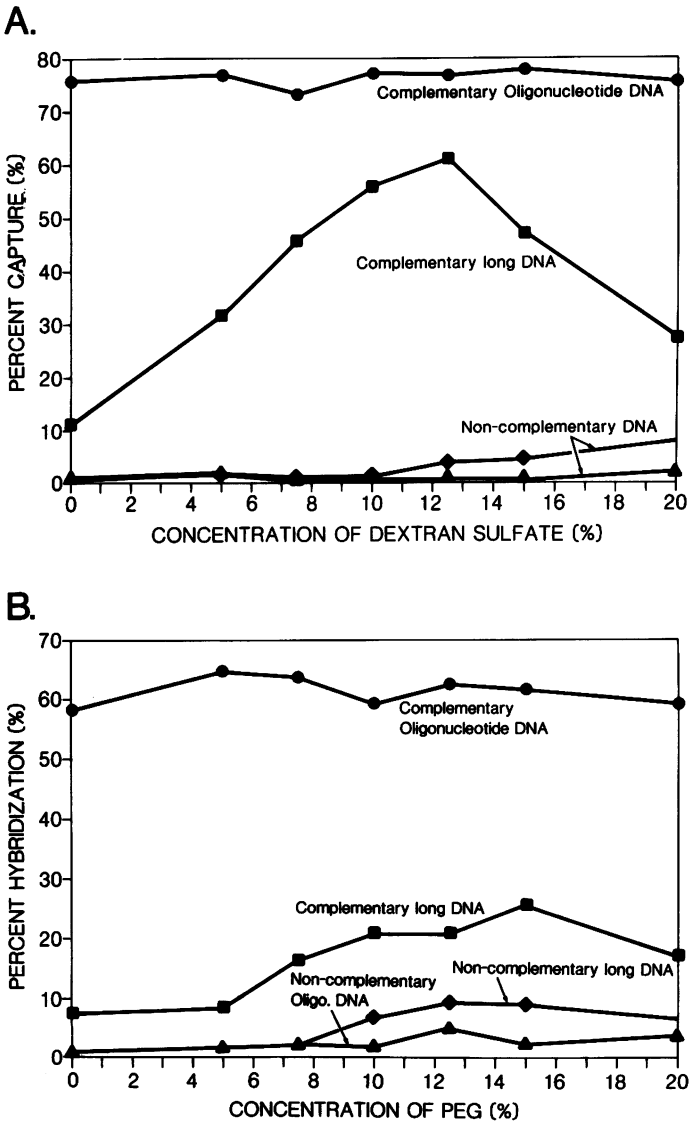

Figure 4. Effects of Volume Exclusion Agents on Support-Bound Oligonucleotide Hybridization. (A) Concentrations of 0-20% dextran sulfate were included in hybridization solutions involving supports which were prehybridized with the same concentration of DS as used in the hybridization solutions. Non-complementary target DNA's were hybridized to the support-bound oligonucleotides as a control. (B) Concentrations of 0-20% polyethylene were included in the hybridization solutions involving supports which were prehybridized with the same concentration of PEG as used in each hybridization experiment.

Kinetics of Support-Bound Oligonucleotide Hybridizations:

The time course for the rate of hybridization of a support-bound oligonucleotide to ^{32}P-labeled target nucleic acids is illustrated in Figure 3. Each hybridization reaction was performed for different lengths of time using 50 mg of supports and a total of 50 fmoles of complementary or non-complementary target DNA. Hybridization of oligonucleotide 85-147 is rapid, requiring no more than 5 minutes to reach peak efficiency (75-80%), with little or no non-specific target binding. The capture of plasmid pTB061B required 60-120 minutes to reach the 45-50% peak efficiency. Interestingly, after 24 hours, the plasmid pTB061B still remains hybridized to the support-bound oligonucleotide. Despite the presence of the complementary strand of pTB061B in the hybridization solution, no strand displacement is observed. Thus, hybridization complexes appear to be stabilized at the support surface by forces in addition to base-to-base hydrogen bonding.

Other Factors Influencing Support-Bound Oligonucleotide Hybridizations:

Experiments were performed to determine which reagent in the prehybridization or hybridization solutions was the most important in producing this optimal hybridization efficiency. Figure 4 shows the results obtained when concentrations of dextran sulfate (DS) and polyethane glycol (PEG) were altered in the support-bound oligonucleotide hybridizations. As compared to hybridization solutions lacking DS, a 4- to 5.5-fold increase in the hybridization capabilities of the support-bound oligonucleotides for pTB061B is observed between concentrations of 8-12% dextran sulfate (Figure 4A). However, no improvement in hybridization is observed for oligonucleotide target 85-147. Non-specific binding of labeled probes increased significantly with concentrations of DS above 10%. Considering the observations of Wetmur (20) that the effectiveness of DS decreases as the lengths of the nucleotide polymers grow shorter, it is interesting to note that in the immobilized oligonucleotide support system, only one member of the hybridization complex need be a long polymer for DS to have a positive effect.

The most noticeable effect of increasing quantities of PEG on the hybridization efficiency is the significant rise in the non-specific background for both oligonucleotide 85-147 and pTB061B

TABLE 2

COMPARISON OF HYBRIDIZATION EFFICIENCIES OF NITROCELLULOSE AND SUPPORT-BOUND OLIGONUCLEOTIDE SYSTEMS

I. Retention Efficiency of Target DNA (pTB061B) by Nitrocellulose Filters

Mock Hybridization Times (hr.)	Immobilized Target Concentration 10 ng	1 ng
1	28 ± 2%	22 ± 4%
24	13 ± 4%	18 ± 2%

II. Detection Efficiency of Immobilized Target DNA[a]

Hybridization Times (hr.)	Immobilized Target Concentration 10 ng	1 ng
1	14.1 ± 0.4%	17.5 ± 0.5%
4	17.9 ± 0.7%	20.0 ± 1%
24	8.9 ± 0.5%	11.1 ± 0.7%

III. Hybridization Efficiency[b]

Type of Support	
Nitrocellulose	Oligonucleotide-Bound Support[c]
50-79%	40-45%

a. Detection efficiency is calculated by:

$$\frac{\text{moles detected}}{\text{moles originally bound to filter}} \times 100\%$$

b. Hybridization efficiency is calculated by:

$$\frac{\text{moles detected}}{\text{moles originally bound to filter}} \times \frac{100\%}{\text{retention efficiency}}$$

c. These figures were obtained from the results reported in Figure 1B for the same target type over the same concentration range.

(Figure 4B). After this background is considered, there is less than a 2-fold net increase in the hybridization signal obtained from plasmid pTB061B. There appears to be no noticeable effect by PEG on the efficiency of hybridization to oligonucleotide 85-147.

Comparison of Nitrocellulose Filter- and Dextran Support-Based Hybridization Systems and Practical Application of the Support-Based Hybridization System:

Two processes were studied in determining the efficiency of a nitrocellulose filter-based hybridization system. The first process is the retention efficiency, which is the measurement of the percent of target nucleic acid which remains immobilized on the filter after the hybridization and washing steps. The second process involves the availability of the immobilized target for hybridization by a labeled probe. These two processes were studied using nitrocellulose filters containing 1 and 10 ng (2-20 x 10^8 molecules) of immobilized pTB061B as target DNA. A retention efficiency of 13-28% for pTB061B was measured, with the lower retention occurring after longer (24-hour) hybridization times (Table 2). The availability of the retained target (pTB061B) was then measured in an experiment using a complementary ^{32}P-end-labeled oligonucleotide probe (85-134). The filters containing target DNA concentrations of one and ten nanograms were hybridized for 1, 4, or 24 hours before being counted. Table 2 shows that the percent detection observed for the 1- and 4-hour filters is similar, but the 24-hour filter measured approximately 50% that of the other two filters. Using both the retention and detection measurements, the hybridization efficiency can be calculated (Table 2). By adjusting for the loss of target DNA from nitrocellulose filters, the hybridization efficiencies of the nitrocellulose system are greater than the support-bound oligonucleotide system. However, with the nitrocellulose system, the ability to detect ^{32}P-labeled target DNA present in the original immobilized sample is seriously compromised by the loss of 80-91% of the target by the end of the hybridization protocol. Consequently, only 8.9% to 20% of the ^{32}P-labeled target DNA is available for detection. By comparison, the support-bound oligonucleotides can detect 40-45% of ^{32}P-labeled target (Figure 1).

DISCUSSION

The investigation of the hybridization properties of oligonucleotide-immobilized supports has resulted in several interesting observations. First, the oligonucleotide-immobilized supports are capable of maximal hybridization efficiencies of 70-80% of the total target DNA for oligonucleotide targets, and 40-50% for denatured, long, double-stranded targets. These limitations on capture efficiencies appear to be independent of the quantity of support-bound oligonucleotide employed to capture a fixed quantity of target (Figure 2) or the concentration of target to be captured by a fixed quantity of support-bound oligonucleotide (Figure 1). These results suggest that the availability of the target in these hybridizations is limiting. This conclusion is confirmed by the results of experiments in which the release of oligonucleotides from the surface of the support were studied. These experiments indicate that part of the inability to capture 100% of the target from solution is attributable to the competition of oligonucleotides freed from the surface with oligonucleotides still attached to the surface of the support (data not shown). Although the amount of released, support-bound oligonucleotides is small (fmoles) in comparison to the amount attached (pmoles), at lower target concentrations, the competing, released oligonucleotides affect the efficiency of hybridization.

The second observation of interest is the stability of the hybridization complex involving the support-bound oligonucleotide and a captured long DNA target. We and others (22-23) have noted that hybridization complexes formed on solid supports between oligonucleotides and longer, complementary DNA molecules are eventually disrupted and the oligonucleotide displaced by the presence of competing complementary strands. Figure 4 indicates that such strand displacement does not occur for hybridization complexes formed between target DNA's and support-bound oligonucleotides. The forces responsible for protecting the captured target DNA strand from displacement appear to involve forces other than base-pair hydrogen bonding. However, the nature of the mechanism stabilizing the hybridization complex to the surface of the support is somewhat curious, since this mechanism must overcome the charge repulsion tendencies of the similarly

negatively charged DNA (due to phosphate backbone) and support surface.

In studies involving the binding of negatively charged human serum albumin to negatively charged latex surfaces, Lyklema (24) has described a mechanism which may explain how a negatively charged surface can bind a negatively charged DNA molecule. In essence, the suggested mechanism involves the formation of an electric-potential well between the two negatively charged surfaces. This well is filled with an accumulation of cations (derived from salt used in the experiment), thus serving to negate the repulsive characteristics of a surface and the attached molecule. Consequently, the hybridization complex may be stabilized at the surface of the supports by virtue of a cation bridge between the DNA and the surface. Such forces binding the DNA target to the support surface can be disrupted by lowering the ionic strength of the hybridization solution or raising the temperature. We have observed that, on the average, 85% of the nucleic acid targets captured by support-bound oligonucleotides can be recovered by heating the complex to 100°C.

The third interesting observation concerns the increased hybridization efficiency obtained by prehybridization of the support-bound oligonucleotide with dextran sulfate. Cohen-Stuart, et al. (25) have noted that macromolecules which are thought to be irreversibly adsorbed can be removed from the support surface by either other displacer macromolecules having stronger adsorption properties or by a large number of smaller, but more tightly adsorbing molecules. Although most of the support-bound oligonucleotides are attached covalently through their 5′ ends, this does not discount the possibility that some of these end-attached oligonucleotides are also adsorbed along their lengths by virtue of the forces previously described. In fact, such adsorption of capture oligonucleotides to the support surface is probably enhanced by virtue of the end attachment as a result of an intramolecular reaction. This adsorption of support-bound oligonucleotides to the surface could consequently interfere with their ability to hybridize target DNA from solution, because the bound oligonucleotide would not be sufficiently free to extend into the hybridization solution. Thus, one func-

tion of DS could be to encourage full extension of the support-bound oligonucleotides into the solution by disrupting the adsorptive forces holding the oligonucleotide to the surface. As noted by Cohen-Stuart, et al. (25), the attached oligonucleotides could be removed from the support surface during prehybridization through the displacement properties of macromolecules whose adsorption characteristics are greater than those of the oligonucleotides. The net result would be to increase the hybridization efficiency of the support-bound oligonucleotides (Table 1).

A second observed function of dextran sulfate has already been described by both Wetmur (20) and Wahl, et al. (26), who have suggested that DS operates by exclusion of the nucleic acids from the volume occupied by the DS, which results in an effective increase in the probe-target concentration and consequently increases the kinetics of hybridization. This effect has been seen by us (Figure 4) and them to be the most pronounced on longer nucleic acids. However, polyethylene glycol (PEG) has also been shown to function as an exclusion reagent (27,28) but, as seen in Figure 4B, it does not have the same effect as DS on hybridization efficiencies. It would seem possible that the negatively charged sulfate groups along the DS are an important functionality of the exclusion reagent, as is its long polymeric structure. The DS may function in two ways during hybridizations involving support-bound oligonucleotides: 1) displacement of adsorbed oligonucleotide probes from the support surface; and 2) by means of volume exclusion, the target and probe concentrations are increased, resulting in improved hybridization efficiencies.

A practical application of ^{32}P-labeling and detection of target DNA using support-bound oligonucleotides can be seen in the use of the Polymerase Chain Reaction protocol (PCR) described by Saiki, et al. (21). This protocol amplifies in vitro the copy number of target DNA prior to detection by use of DNA polymerase-dependent, oligonucleotide-directed replication. Any such amplified target DNA can be made radioactive by the use of radioactively labeled nucleotides, for incorporation during the last round of amplification. This labeled target DNA can be captured by the support-bound oligonucleotides in a manner identical to that described in Materials and Methods.

In summary, the covalent end-attachment of oligonucleotides to dextran supports provides a means of rapidly and efficiently purifying long or short target sequences of interest. The hybridization capacity of the support-bound oligonucleotide is large, but it is chiefly the formation of structures by the target that limits the extent of capture. As the practical need for detection of low copy-number nucleic acid sequences increases, the ability to separate and concentrate these rare target sequences from a pool of background nucleic acids will become essential. An oligonucleotide-based affinity format can provide the specificity required for this purification, as well as simplicity of use.

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*To whom correspondence should be addressed

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