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Oligonucleotide adsorption to artificial surfaces

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Abstract

A necessary step in the development of a candidate drug involves characterization of the drug's interactions with the natural and synthetic surfaces it encounters. In this preliminary study, the time-, solvent-, surface-, and cation-dependency of oligodeoxynucleotide (ON) adsorption was examined by measuring loss of a radiolabeled phosphodiester ON from either cell growth media or distilled, deionized water (with or without different added cations) as a function of time and surface. As expected, ON adsorption was dependent on all the factors examined. Either silica coating of surfaces or the addition of cations, EDTA or heat-inactivated serum, may be useful techniques for reducing ON adsorptive losses.

Keywords: Adsorption; Antisense; Binding; Cation; Drug development; Oligonucleotide

Oligodeoxynucleotides (ONs) represent a growing class of new therapeutic agents designed to specifically down-regulate gene expression (Wu-Pong, 1994). These polyanionic macromolecules enter cells and bind matching DNA or RNA sequences resulting in inhibition of expression of the target gene. Extensive in vitro testing of ONs has recently resulted in clinical testing of new ON drug candidates.

Synthesis and in vitro testing of ONs necessarily involve a variety of solvents, tubes and pipettes of different compositions to store, transfer or test ONs. However, data regarding ON adsorption to artificial surfaces has not yet been published despite the obvious implications in data interpretation and adsorptive loss during clinical use. Characterization of the interaction of these macromolecules with artificial surfaces will become increasingly important during ON formulation and manufacturing. In this preliminary study, ON adsorption to hydrophilic (e.g. borosilicate glass) and hydrophobic (e.g. Teflon) surfaces were examined as a function of time, solvent, surface, and cation added using a representative phosphodiester ON. As anticipated, ON adsorption was time-, surface, cation- and solvent-dependent.

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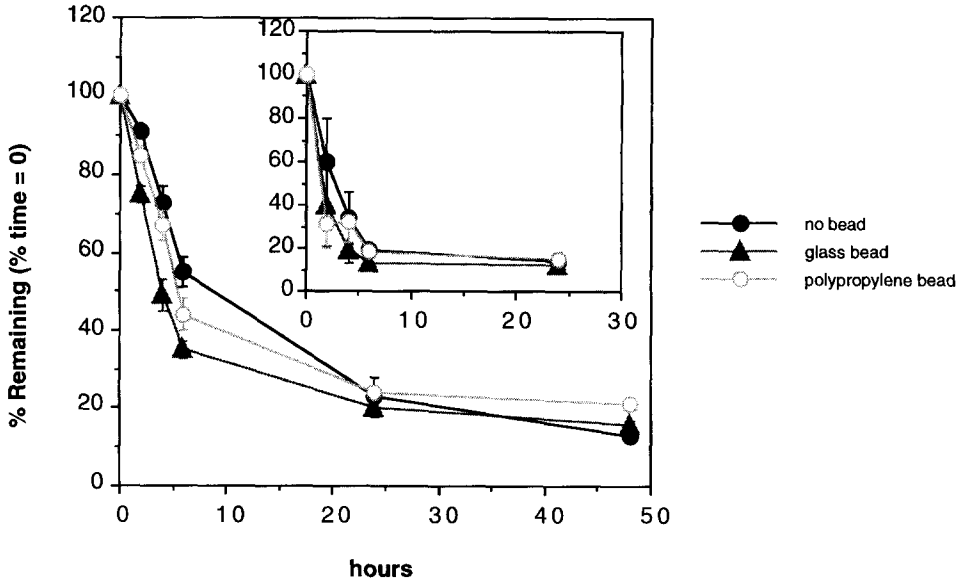

Fig. 1. Radiolabeled ON was added to replicate borosilicate tubes with or without glass or polypropylene beads at time = 0, and incubated in media with (insert) or without continuous shaking. Tubes were incubated at ambient temperature for up to 48 h, then quadruplicate samples were removed and counted in scintillation cocktail. Data represents the mean of 2 experiments ± standard error.

Therefore, ON losses should be quantified at each step during testing, formulation and manufacture and appropriate steps should be taken to minimize losses due to adsorption.

A c-myc antisense phosphodiester ON (5'GAA GTT CAC GTT GAG GGG CAT) (Oligos Etc., Wilsonville, OR), was 5' end-labeled with γ ^{32}P-ATP by standard methods (Sambrook et al., 1989). The resulting ^{32}P-ON predominantly results in a single radioactive species, although a high molecular weight species is sometimes detected (Wu-Pong et al., 1992) possibly resulting from ON complexation. Either cell growth media (Minimum Essential Media, Alpha Modification, Sigma, St. Louis, MO) or deionized, distilled water was mixed with up to 20 mM $CaCl_2$, $MgCl_2$, $NiCl_2$, or $LaCl_2$. ^{32}P-ON was added to the solution (specific activity of 10^5–10^6 cpms/ml) such that the final concentration was either 100 pM or 10 μM. At time (t) = 0, 200 μl of the ON solution was added to two replicate borosilicate glass (Fisher Scientific, Fairlawn NJ, 10 mm × 75 mm) or 1.5 ml polypropylene microcentrifuge (Fisher Scientific No. 0540723A) tubes with or without a Teflon (polytetrafluoroethylene), Delrin (acetal), nylon, polypropylene (1/8 inch diameter, Small Parts, Miami, FL), or borosilicate glass (8 mm, VWR Scientific, Bridgeport NJ) bead such that the bead was completely covered. Samples were then incubated at ambient temperature (23–25°C) for up to 48 h. Quadruplicate 5 μl samples (ranging from approximately 200–2000 cpm over background) were removed from each tube at increasing time, mixed with scintillation cocktail, then counted in a scintillation counter. Data was calculated as % of cpms at $t = 0$. Variability in $t = 0$ samples resulted from ON adsorption during sample preparation; data with outlier values at $t = 0$ were discarded. All materials (tubes, beads) used in this study were used as supplied. The ON was undegraded under the conditions used in this study (data not shown).

ON adsorption from low (100 pM) starting concentrations to the borosilicate glass tube was time-dependent (Fig. 1). Adsorption was inhibited by adding either 10% heat-inactivated serum (data not shown), calcium, nickel (Table 1), or EDTA (Fig. 2) to the media prior to incubation with the

Table 1
ON (100 pM) remaining in solution in borosilicate or polypropylene tubes

Cation added	Borosilicate tubes[a] (% remaining)[b]				Polypropylene tubes[a] (% remaining)[b]			
	Media		Water		Media		Water	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
None	30 ± 18	22 ± 11	97 ± 1	98 ± 1	78 ± 7	67 ± 14	80 ± 11	68 ± 16
Ca 10 mM	106 ± 12	142 ± 32	76 ± 9	91 ± 23	116 ± 18	107 ± 13	ND	ND
20 mM	94 ± 1	98 ± 23	ND	ND	98 ± 13	100 ± 10		
Mg 10 mM	25 ± 12	22 ± 8	83 ± 5	71 ± 12	83 ± 8	50 ± 12	97[c]	98[c]
20 mM	25 ± 7	24 ± 4	92 ± 4	91 ± 3	97 ± 2	61 ± 25	121 ± 19	130 ± 21
Ni 10 mM	130 ± 38	48 ± 43	85 ± 8	81 ± 7	80 ± 17	93 ± 16	99 ± 21	99 ± 11
20 mM	135 ± 2	70 ± 24	88 ± 12	93 ± 29	59 ± 7	92 ± 4	68 ± 14	79 ± 14
La 10 mM	58 ± 7	95 ± 54	85 ± 13	85 ± 13	47 ± 13	210 ± 38	63 ± 6	66 ± 4
20 mM	37 ± 23	47 ± 13	86 ± 1	95 ± 27	37 ± 8	58 ± 16	64 ± 1	62 ± 9

[a]% Time = 0, $n = 2–4$.
[b]Mean ± standard error, measurements taken from time the ON was added to the tube ± bead.
[c]Second data point excluded due to criteria described in Methods.

ON. In addition, adding EDTA to the media after the ON was adsorbed to the tube reversed ON adsorption (data not shown).

The effect of the composition of the adsorbing surface was also examined. When incubated in media and glass tubes for 48 h, 78% of the ON was lost due to adsorption compared to 30% lost when incubated in polypropylene tubes (Table 1). The rate of ON adsorption was decreased by coating glass tubes with silica (Sambrook et al., 1989) prior to adding the ON, although significant adsorption still occurred (40% lost from solution after 48 h, S.E. = 1, $n = 2$).

As anticipated, ON adsorption was also solvent-dependent, since generally the solvent determines the tendency of the solute (ON) to escape from the solvent. ON adsorption to glass tubes decreased from 78% when incubated in media, to 2% when incubated in water. Adsorption to polypropylene tubes remained at 20–30% regardless of solvent (Table 1). Addition of different cations to either media or water had varying effects on ON adsorption, increasing adsorption in some cases, but decreasing adsorption in others (Table 1).

Adsorption was also dependent on the interaction between surface and solvent. For example, when incubated in water, the ON adsorbed only to the glass bead. When calcium was added to the water, the ON adsorbed to all beads (Fig. 2). ON adsorption to different glass surfaces (glass bead vs. glass tube) also differed (Figs. 1 and 2), probably due to variations in the composition of the materials used to manufacture each glass product (Holloway, 1973).

ON adsorption to plastic and glass was also examined after incubation using a biologically relevant ON concentration, 10 μM, a concentration commonly used in cultured cells to down-regulate gene expression (for review see (Wu-Pong, 1994)). Adsorption resulting from incubation with 10 μM ON was only 3-fold less than incubations using a 100-fold lower ON concentration (100 pM) (Tables 1 and 2).

Adsorption occurs if (a) the solute and surface have chemical moieties which are capable of interacting or (b) if the solute is surface active and is interacting with a large surface area (nonspecific adsorption). Polymer adsorption can be quite complex and is therefore not well understood, in part because of the possibility of a variety of adsorption mechanisms. This study reveals that definitive generalizations regarding ON adsorption are not easily made, since ON adsorption is a function of the interaction between time, solvent, surface, and added cation (Table 1, Fig. 1).

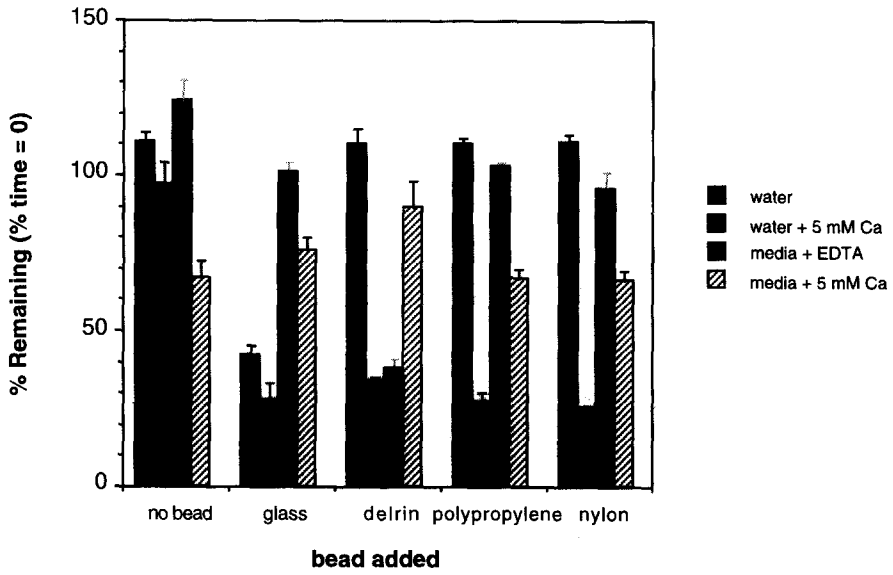

Fig. 2. Radiolabeled ON was added to replicate borosilicate tubes with or without glass, Delrin, polypropylene or nylon beads at time = 0, and incubated in either water or media ± 5 mM calcium chloride or EDTA. Tubes were incubated at ambient temperature for 24 h, then quadruplicate samples removed and counted in scintillation cocktail. Data represents the mean of 4 experiments ± standard error.

Multivalent cations appear to play a role in ON adsorption. ON adsorption tends to be more extensive when incubated in growth media, which contains a variety of mono- and divalent cations (Table 1). In addition, ON adsorption is reversed when EDTA is added to the media (Fig. 2) suggesting that cations mediate ON adsorption to the artificial surfaces, possibly by charge neutralization of the anionic ON. However, adding cations to water or media did not uniformly increase ON adsorption to the surfaces tested (Table 1, Fig. 2) and, in fact, had variable effects on ON adsorption to both tube and bead (Table 1). Thus, charge-neutralization is an overly simplistic model which alone cannot account for ON adsorption.

Clearly, the mechanism of ON adsorption to artificial surfaces is dependent on many factors as demonstrated in this preliminary study. The complex, time-dependent interaction between surface and solvent makes predicting adsorption outcomes impossible at this time. Therefore, ON adsorption should be quantitated at every step of ON synthesis, storage, and testing to ensure the presence of expected concentrations of ON, or to modify existing conditions to minimize costly ON loss due to adsorption. Addition of heat-inacti-

Table 2
ON adsorption (10 μM) in borosilicate or polypropylene tubes

Time	Borosilicate tubes[ab] (% remaining)		Polypropylene tubes[ab](% remaining)	
	Media	Water	Media	Water
Day 1	82% ± 6	97% ± 4	89% ± 1	98% ± 8
Day 2	81% ± 5	96% ± 2	84% ± 2	97% ± 1

[a]Mean ± S.E.
[b]% Time = 0, $n = 2$.

vated serum, multivalent cations, or EDTA to ON-containing solutions may be useful for reducing ON adsorptive losses. Silica coating of surfaces may also decrease the rate of ON adsorption and may therefore be preferable to the adding cations or serum to the ON solution. If significant losses cannot be avoided, the apparent ON concentration must be determined when testing ONs in vitro or in vivo.

Acknowledgements

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