# THE BINDING OF RNA TO VARIOUS CELLULOSES

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SUMMARY

Several celluloses were tested for their ability to bind homonucleotide oligomers and natural RNAs. Columns of these celluloses were run under conditions favoring hydrogen bond formation (neutral pH and high salt concentration). The materials binding at the high salt were released by eluting the columns with solutions of lower ionic strength. The binding to unesterified cellulose was much less specific than that found with celluloses esterified with oligo(dT) or oligo(dC) residues. The different cellulose preparations were quite different in their ability to bind the oligonucleotides and the RNAs. These variations suggested that an impurity present in the cellulose in varying amounts rather than the cellulose itself, is responsible for the binding properties of these samples. Treatment of the celluloses with sodium bisulfite reduced the amount of poly(A) binding which suggests that the binding is due to a lignin-like contaminant.

Within the last few years there have been a number of methods published (1-5) using oligo(dT)-cellulose for the isolation of heterogeneous nuclear and messenger RNA from eukaryotic cells. The isolation of these RNAs depends on the presence of a poly(A)-rich region in these molecules (6-9). This poly(A) "hybridizes" with the oligo(dT) esterified to the cellulose and is retained on the column.

More recently, reports of the purification of messenger RNA from eukaryotic cells using unesterified cellulose have appeared (10-12). Since we have recently shown that brain "messenger" binds to oligo(dT)-cellulose, but not to oligo(dC)-cellulose (13), the reports of messenger binding to untreated cellulose seemed difficult to understand. In order to determine if poly(A) binding is a general property of cellulose, a number of different celluloses were tested for their ability to bind poly(A) as well as poly(I), poly(U), and different types of natural RNA. The results indicate that different celluloses have large variations in their ability to bind homopolymers and that this binding is not speci-

fic for poly(A), but also extends to at least one other polypurine, poly(I). The data suggest that the binding is due to a lignin-like impurity in some of the celluloses.

## MATERIALS AND METHODS

CF-11 and CC-41 celluloses were obtained from Whatman. Sigmacell type 38 cellulose was purchased from Sigma Chemical Co. Poly(A), poly(U), and poly(I) were obtained from Calbiochem. The poly(A) used for the experiment shown in Table 3 was obtained from Miles Laboratories. The radioactive homopolymers were products of Schwartz Bio-Research, Inc.

Oligo(dT)- and oligo(dC)-celluloses were prepared from CF-11 Whatman cellulose by the method of Gilham (14). The columns were made by pouring 100 mg portions of the various celluloses into Pasteur pipettes plugged with glass wool. The samples were applied in minimum volumes of Tris-HCl buffer (0.01 M, pH 7.4) containing 0.5 M KCl. The column was washed with 3 ml of the same buffer. Then the columns were washed with 3 ml of 0.01 M Tris-HCl buffer, pH 7.4, and, finally, with 3 ml of deionized water. The material washed off by the low salt buffer and the water washes was considered to be the amount bound by the column. The columns were regenerated for use by washing with 2 ml of 0.1 M KOH and reequilibrated with the starting 0.5 M salt-containing starting buffer.

The RNA and the homopolymers were measured by determining the $A_{260}$ with a Gilford spectrophotometer. The radioactive RNA or the radioactive homopolymers were quantitated by counting in a Packard 4000 series scintillation spectrometer using Aquasol (New England Nuclear) as a counting solute.

The bulk brain RNA was prepared as previously described (13). The brain ribosomal RNA was prepared from brain ribosomes obtained by the method of Murthy (15). The RNA was extracted as described by Jarlstedt and Hamberger (16). The preparation was freed of messenger by passing it over a large column of oligo(dT)-cellulose. E. coli ribosomal RNA was a gift from Dr. Alan Peterkofsky.

The cellulose was treated with Pronase, RNase, or DNase by incubating 1 mg of the appropriate enzyme with 100 mg of cellulose in a 1 ml volume for 60 min at room

temperature. Treatment of the cellulose with $NaHSO_3$ involved autoclaving 500 mg portions of the cellulose in 5 ml of 1 M $NaHSO_3$ overnight and then filtering the cellulose while still hot. The exact amount of cellulose in each of the columns was measured by the dry weight of an appropriate portion of the material used to pour the column.

## RESULTS

The various preparations examined have different binding capacities for the homopolymers (Table 1). As can be seen, the substituted celluloses bound the expected homopolymer; oligo(dT)-cellulose bound poly(A), and oligo(dC)-cellulose bound poly(I). Neither column retained poly(U). The CF-11 cellulose which had been run through the condensing procedure in the absence of any nucleotide bound about the same amount of the polymers that the untreated CF-11 cellulose bound, showing that the treatment itself did not change the binding properties of the cellulose.

The several celluloses, CF-11, CC-41, and Sigmacell 38, had widely different binding properties. None of them bound poly(U) but Sigmacell had about 5x the poly(A) capacity of CF-11. The binding was not specific, however, because the Sigmacell also bound about twice as much poly(I) as did the CF-11. None of the unesterified celluloses were equal in capacity for poly(A) to the oligo(dT)-cellulose but the Sigmacell cellulose bound more poly(I) than the CF-11 specifically esterified for that purpose. When small amounts of labelled polymers were added to the columns the results were consistent with the data in Table 1. The oligo(dT)-cellulose column bound essentially all the radioactive material showing that these columns will hold all the appropriate polymer passed over them until the capacity of the column is exceeded.

When the bulk RNA from the brain was passed over the columns, some material was retained (Table 2). Labelled brain RNA has been shown to contain material which binds to oligo(dT)-cellulose (13). Clearly, this material does not bind to oligo(dC)-cellulose nor to unsubstituted CF-11 cellulose. But the CC-41 and Sigmacell 38 bound significant quantities and the binding was in rough propor-

Table 1. Binding of Homopolymers to the Different Celluloses

| Column | Poly(A)[1] $A_{260}$ units bound | Poly(I)[2] $A_{260}$ units bound | Poly(U)[3] $A_{260}$ units bound |
|---|---|---|---|
| Oligo(dT)-cellulose (CF-11) | 3.56 | 0.90 | 0.33 |
| Oligo(dC)-cellulose (CF-11) | 0.60 | 1.32 | 0.36 |
| Treated cellulose (CF-11) | 0.48 | 1.06 | 0.21 |
| Untreated cellulose (CF-11) | 0.54 | 1.05 | 0.22 |
| Untreated cellulose (CC-41) | 1.26 | 1.33 | 0.37 |
| Untreated cellulose (Sigmacell-38) | 1.98 | 1.83 | 0.28 |

[1]Added 4.52 $A_{260}$ units to each column
[2]Added 2.40 $A_{260}$ units to each column
[3]Added 4.12 $A_{260}$ units to each column

Table 2. Binding of RNA to the Different Celluloses

| Column | Labelled brain RNA[1] cpm bound | Brain ribosomal RNA[2] $A_{260}$ units bound | *E. coli* ribosomal RNA[3] $A_{260}$ units bound |
|---|---|---|---|
| Oligo(dT)-cellulose (CF-11) | 3,411 | 0.38 | 0.34 |
| Oligo(dC)-cellulose (CF-11) | 492 | 0.23 | 0.32 |
| Treated cellulose (CF-11) | 519 | 0.11 | 0.31 |
| Untreated cellulose (CF-11) | 561 | 0.37 | 0.30 |
| Untreated cellulose (CC-41) | 1,107 | 0.34 | 0.28 |
| Untreated cellulose (Sigmacell-38) | 1,890 | 0.20 | 0.19 |

[1] Added 9850 cpm, 3.74 $A_{260}$ units to each column
[2] Added 7.63 $A_{260}$ units to each column
[3] Added 7.65 $A_{260}$ units to each column

Table 3. Effect of $NaHSO_3$ Treatment on the Binding of Poly(A) to the Different Celluloses

| Column | Autoclaved[1] with $H_2O$ | Autoclaved[1] with 1 M NaCl | Autoclaved[1] with 1 M $NaHSO_3$ |
|---|---|---|---|
| | Poly(A)[2] $A_{260}$ units bound | | |
| Whatman CF-11 | 0.46 | 0.40 | 0.21 |
| Whatman CC-41 | 2.57 | 2.58 | 0.64 |
| Sigmacell-38 | 3.23 | 3.24 | 0.92 |

[1] 500 mg of the cellulose was autoclaved (20 lbs, 121°) for 16 hours in 5 ml of the appropriate solution, filtered while still hot, and washed with hot water and with ethanol.

[2] Added 3.60 $A_{260}$ units to each column

tion to the binding of poly(A) by these materials. Neither brain nor E. coli ribosomal RNA bound to any of the preparations used.

Treatment of the Sigmacell 38 cellulose, the material with the highest binding capacity for poly(A), with either Pronase, RNase, or DNase did not lower its ability to bind the homopolymer. On the other hand, treatment with $NaHSO_3$ lowered the capacity of this cellulose to bind poly(A) by more than 70% (Table 3).

## DISCUSSION

The data presented here confirms the reports that untreated Sigmacell 38 cellulose will bind poly(A) (11) and messenger RNA from eukaryotes (12). These data have been confirmed exactly and directly by Drs. Haim Aviv and Philip Leder (personal communication). The binding appears to be due to the presence of the poly(A), but does not seem to be specific, since poly(I) binds to the same cellulose preparations. The use of the appropriate untreated cellulose preparations probably is of value in the purification of messenger RNA since ribosomal RNA does not bind at all to these celluloses. However, oligo(dT)-cellulose binding would seem to be the method of choice because of its specificity and because its capacity is substantially greater.

The binding of poly(A) sequences to untreated celluloses does not seem to be due to the intrinsic properties of the cellulose itself. The observation that treatment with $NaHSO_3$ lowers the capacity suggests that an impurity in the preparation is responsible for the binding. Since treatment with $NaHSO_3$ is one of the classic ways to remove lignin from wood pulp (17), the data indicate that the binding is due to lignin itself or to some lignin-like material. Lignin is a polymer of aromatic residues, and, thus, is an appropriate candidate for the binding of polynucleotides, more specifically, polypurines. The amount of lignin, or of lignin-like materials, contaminating the various celluloses determines the ability of these preparations to bind messenger RNA. The capacity of these materials to bind polypurines must be enormous since the contaminations found in these highly purified samples should not be above trace levels.

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