Discussion

The reaction described gives a comparatively high yield of a xanthine adduct with tryptophan. The structure is drastically altered from the flat indole system to a highly three-dimensional indolenine, which has an analogy to the indolenine alkaloids condifoline and tubifoline (Klyne *et al.*, 1966). In parallel to this substitution reaction several other products, probably oxidation products resulting from the oxidative reactivity of 3-acetoxyxanthine (G. Stöhrer, in preparation), are formed from tryptophan. The formation of these so far uncharacterized products is favored at physiological pH (Figure 2).

Preliminary metabolic experiments showed that compounds IIIa and IIIb cannot be found in alkaline protein digests, but they are not stable under these conditions. The results show that IIIa may be present in the urine to an extent of not over 0.25% of the total radioactivity, but this cannot yet be confirmed by a suitable derivatization.

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Adsorption of Polyadenylate and Other Polynucleotides to Unmodified Cellulose†

Paul A. Kitos and Harold Amos*

ABSTRACT: Under conditions of high ionic strength, single-stranded polyadenylate (poly(A)) binds to microcrystalline cellulose or a contaminant thereof and can be eluted by lowering the ionic strength of the solution. Single-stranded DNA behaves like poly(A) in these respects but pyrimidine polyribonucleotide homopolymers do not. Likewise, poly(A)·poly(U) hybrids have no affinity for cellulose preparations. In high ionic strength solution excess poly(U) removes most of the bound poly(A) from the poly(A)–cellulose complex but does not affect the bound single-stranded DNA. Poly(U) can be used to obtain substantial separation of poly(A)-containing RNA from nucleic acid mixtures which contain single-stranded DNA by selectively eluting the poly(A)-containing material from the cellulose-bound mixture of the two. An explanation of the binding phenomenon is proposed.

In high ionic strength solution polyadenylate (poly(A)) and other single-stranded purine polyribonucleotide homopolymers adsorb to unmodified and certain partially esterified celluloses. At pH values greater than 6.2 they can be eluted from the adsorbing surface by decreasing the ionic strength of the environment (Sullivan and Roberts, 1971, 1973; Kitos *et al.*, 1972; DeLarco and Guroff, 1973; Lee *et al.*, 1971; Edmonds *et al.*, 1971). Under similar conditions DNA also binds to cellulose and certain of its derivatives (Nygaard and Hall, 1964; Gillespie, 1968; Kitos *et al.*, 1972). Pyrimidine ribonucleotide homopolymers and heteropolymeric RNA adsorb either poorly or not at all (Kitos *et al.*, 1972).

Poly(dT)–cellulose (Edmonds *et al.*, 1971; Aviv and Leder, 1972; Swan *et al.*, 1972), Millipore filters (Lee *et al.*, 1971; Rosenfeld *et al.*, 1972; Yogo and Winner, 1972), and unesterified cellulose (Sullivan and Roberts, 1971, 1973; Kitos *et al.*, 1972; Schutz *et al.*, 1972, 1973) have been used for isolating poly(A)-containing RNAs from complex mixtures. Nitrocellulose membranes have been used extensively in hybridization studies (Gillespie and Spiegelman, 1965; Gillespie,

† From the Department of Biochemistry, The University of Kansas, Lawrence, Kansas 66044 (P.A.K.), and the Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115 (H.A.). *Received May 24, 1973.* This investigation was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (Grant Number AI-00957), the American Cancer Society, Inc. (Grant Number NP-51F), and the American Heart Association (Grant Number 70-829). This investigation was also supported in part by National Institutes of Health Fellowship Number 6 FO3 CA-52,055-01 from the National Cancer Institute, and a grant from the National Science Foundation (Number GB13924).

1968). Under certain dielectric circumstances cellulose is useful in the fractionation of complex RNA mixtures (Barber, 1966; Franklin, 1966; Kronenberg and Humphreys, 1972). Several other polynucleotide-adsorbing systems, not involving cellulose or its derivatives, have been used in the isolation of purine nucleotide polymers (Lim and Canellakis, 1970; Sheldon *et al.*, 1972).

In this paper we report the effects of certain other homopolymeric RNAs on the binding of poly(A) to cellulose and describe a method by which cellulose-bound DNA and most of the poly(A) in commercial preparation can be separated.

Materials and Methods

The cellulose used for all the binding studies reported here was Sigmacell type 38, purchased from the Sigma Chemical Co., St. Louis, Mo. Before use it was suspended in water at a known weight per unit volume and portions of the suspension, each containing 60 mg of cellulose, were introduced into 6-in. diSPo Pasteur pipets (5-mm i.d., Scientific Products Co., Waltham, Mass.) which had been fitted at the conical end with a glass fiber filter. The cellulose was allowed to pack in the column using only the gravity flow rate (maximum pressure head, about 5 ml of water). The column was then washed with water followed by excess sample–application buffer (buffer H). This method of preparation of the columns enabled us to obtain highly reproducible flow rates and elution patterns.

High ionic strength buffer (buffer H, μ = 0.51) containing 10 mM Tris-HCl (pH 7.6), 500 mM KCl, and 0.2 mM $MgCl_2$ was used to dissolve various polynucleotides and to wash the cellulose columns both before and after each sample application. In our previous studies (Kitos *et al.*, 1972) we had used deionized-redistilled water as the low ionic strength solvent system for the elution of poly(A) from the cellulose. However, we found that poly(A) would remain bound to the cellulose even at low ionic strength if the pH was below 6.2. Since it is well known that the pH of distilled water can be variable and difficult to control, a low-salt buffer was found to be more reliable. Therefore, in the present study buffer K containing 0.5 mM Tris-HCl (pH 7.6), 0.5 mM KCl, and 0.01 mM $MgCl_2$ was used as the low ionic strength elution solution. The ionic strength (μ) was 0.001.

The polyadenylate (poly(A)) used in these studies was obtained as the potassium salt from Miles Laboratories, Elkhart, Ind. The polydisperse material was characterized by the supplier as having a minimum molecular weight of 10^5 daltons, determined by gel filtration, an $s_{20,w}$ of 6.80 S in a 0.1 M NaCl–0.05 M phosphate buffer (7.0), an organic phosphorus content of 2.42 μmol/mg, an E_p at 260 nm of 9.81×10^3 at pH 7.0, and a pK of approximately 6.0. The [^{3}H]poly(A) was also obtained from Miles Laboratories but it had a minimum molecular weight of 5×10^4 daltons, by gel filtration. It contained 10.8 μg of polymer/μCi of ^{3}H. The polyuridylate (poly(U)) (Miles) was obtained as the ammonium salt and had a minimum molecular weight of 10^5 daltons by gel filtration, an $s_{20,w}$ of 7.43 S in 0.1 M NaCl–0.05 M phosphate buffer (pH 7.0), an organic phosphorus content of 2.60 μmol/mg, and an E_p at 260 nm of 9.7×10^3 at pH 7.0. Polycytidylate (poly(C)) (Miles) was obtained as the potassium salt.

The calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.) was denatured by heating in a dilute salt solution (buffer I, 10 mM Tris-HCl–10 mM KCl–0.2 mM $MgCl_2$, pH 7.6) to 96° for 5 min followed by rapid chilling, and was then diluted for use in buffer H.

Unless otherwise stated the cellulose chromatography was carried out by adding the sample in 1 ml to the column. This volume was allowed to percolate through the cellulose during which time all the effluent liquid was collected in a small test tube. The receiving vessel was then changed and the next 1 ml of liquid (usually buffer H) was added to the column. In these small columns the void volume was only a few drops and therefore was small compared with the applied volume of 1 ml. Each aliquot of appropriate solution, when added to the column, was allowed to pass completely into the cellulose bed before the next unit was added.

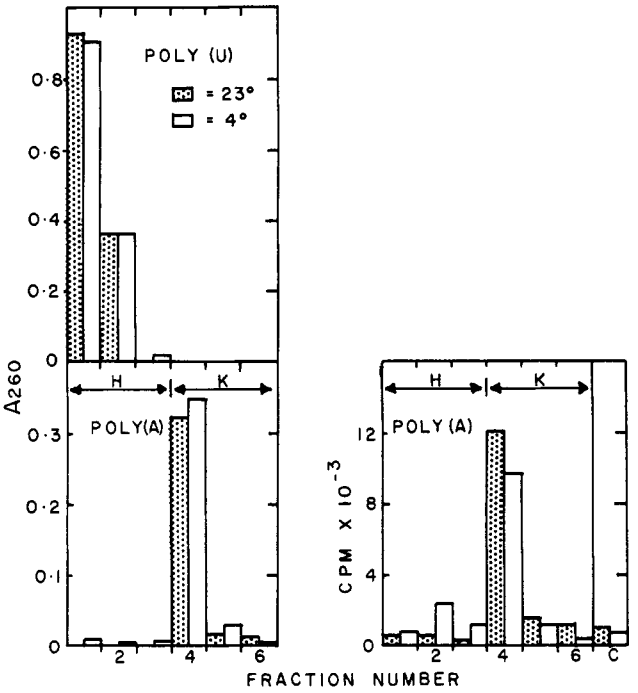

FIGURE 1: The binding of poly(A) and poly(U) to cellulose. [^{3}H]-Poly(A) (0.420 A_{260} unit, 17,350 cpm) or poly(U) (1.284 A_{260} units, nonradioactive) was added to a 60-mg cellulose column in 1 ml of buffer H. The column was then washed with two successive 1-ml portions of buffer H and three successive portions of buffer K. Each 1-ml fraction was collected separately and its absorbance at 260 nm and, in the case of the poly(A) study, its radioactivity were determined. Identical studies were carried out at room temperature (23°) and refrigerator temperature (4°). The amount of uv-absorbing and radioactive material in each fraction is represented in this chart. The stippled bars represent values obtained in the room temperature study. Fractions 1–3 contain the high-salt buffer (H) and fractions 4–6 contain the low-salt buffer (K). The column labeled "C" designates the amount of radioactive material still adhering to the cellulose even after the third buffer K fraction was collected (see legend to Table I).

The absorbance at 260 nm and the radioactivity of all starting samples and column fractions was measured. Whenever hybrid polynucleotides were prepared the hypochromicity was determined by measuring the absorbance of both the individual polynucleotide solutions and the mixture. Hybrids between poly(A) and poly(U) were annealed at room temperature for at least 10 min before use. The radioactivity of both the starting material and the column fractions was measured by drying defined portions of them on 25-mm diameter glass filter disks and counting in plastic vials using a toluene-based scintillation cocktail (4 g of Omnifluor/l. of toluene) and a liquid scintillation spectrometer at peak efficiency settings. To determine the amount of radioactive material which does not elute from cellulose even at low-salt concentrations the cellulose was quantitatively extruded from the column into a plastic scintillation vial using a small amount

TABLE I: Adsorption of Single- and Double-Stranded RNA Homopolymers to Cellulose.[a]

Added to the Cellulose Column			Effluent from the Column in				cpm Retained by Cellulose after Buffer K
			Buffer H (Not Adsorbed)		Buffer K (Adsorbed)		
Component(s)	A_{260}	cpm	A_{260}	cpm	A_{260}	cpm	
Poly(U)	0.666		0.655 (98)[b]		0 (0)		
Poly(U)	1.284		1.287 (100)		0 (0)		
Poly(U)	2.722		3.014 (110)		0 (0)		
Poly(A)	0.420	17,450	0 (0)	1,350 (8)	0.350 (83)	14,640 (84)	946 (5)
1 + 4[c]	0.690	16,040	0.598 (87)	14,160 (88)	0.068 (10)	2,540 (16)	100 (1)
2 + 4	1.170	15,340	0.940 (80)	12,930 (84)	0.050 (4)	1,460 (10)	49 (0)
3 + 4	2.680	14,570	2.856 (107)	14,300 (98)	0.045 (2)	1,261 (9)	45 (0)

[a] The poly(A) used in this study was prepared by mixing a small amount of ^{3}H-labeled poly(A) with a somewhat larger amount of unlabeled poly(A) in buffer H. The solutions of poly(U) were also in buffer H. Predetermined amounts of each homopolymer solution were mixed and allowed to stand for at least 10 min before being used chromatographically as poly(U)·poly(A) mixtures. In each case the sample was applied in 1 ml to a Pasteur pipet cellulose column. The column was then washed successively at room temperature with two 1-ml portions of buffer H and then with three 1-ml portions of buffer K. During the entire operation the column effluents were collected in 1-ml fractions. The absorbance at 260 nm and the radioactivity of each fraction were measured. The column effluent values reported in this table are summations of the values for the three 1-ml fractions which were collected during passage of the specified buffer. [b] Values in parentheses are percentages of the amount added to the cellulose column. [c] The poly(U)·poly(A) mixture used in these studies contained the amount of poly(A) indicated on line 4 plus the amount of poly(U) indicated on line 1, 2, or 3. The uv absorbancies of these solutions (lines 5, 6, and 7) are less than the sum of the absorbancies of their components due to a strong hypochromic effect.

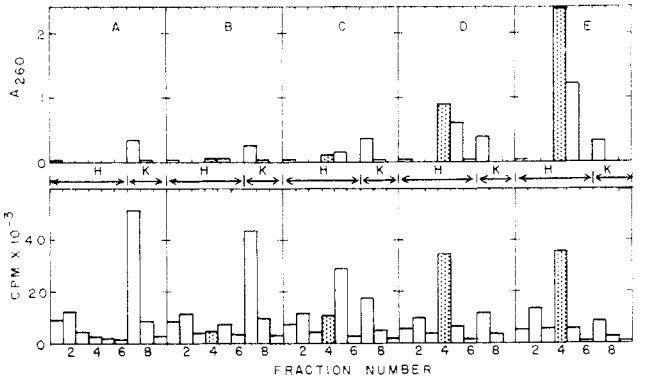

FIGURE 2: The binding of poly(A) to cellulose and its elution with poly(U). Each of five replicate samples of poly(A) (0.469 A_{260} unit, 112,720 cpm) in 1 ml of buffer H was adsorbed to a 60-mg cellulose column in a Pasteur pipet. The columns were then washed with five 1-ml portions of buffer H followed by three 1-ml portions of buffer K. To four of these columns (B–E) poly(U) was added in the 1-ml addition of buffer H indicated by stipling at fraction 4. The amount of poly(U) added to each column was as follows: column A, none; column B, 0.469 A_{260} unit; column C, 0.893 A_{260} unit; column D, 2.148 A_{260} units; and column E, 4.296 A_{260} units. The absorbance and radioactivity of each collected fraction are expressed by the bars in the figure. The regions indicated by H and K represent the fractions obtained during the passage of buffers H and K, respectively.

of water. The aqueous slurry was dried in a 60° oven and 10 ml of the scintillation liquid was added to it.

Results

Cellulose Adsorption of Poly(A) and Poly(A)·Poly(U) Hybrids. Microcrystalline, unmodified cellulose adsorbs poly(A) with rather high capacity from aqueous solution of high ionic strength (Kitos *et al.*, 1972). Indirect evidence suggests that the process depends upon the molecular conformation of poly(A). We have examined the binding characteristics of single-stranded poly(A) and of poly(A) in double-helical (poly(A)·poly(U)) or triple-helical (poly(A)·2 poly(U)) structures (Thrierr and Leng, 1972). In hybrid association with poly(U), poly(A) has little or no affinity for cellulose. Poly(A)·poly(U) hybrids in high ionic strength buffer (buffer H) were passed through small cellulose columns. To aid in distinguishing between the polynucleotide components of the effluent liquid the poly(A) used in the study was tritium labeled. Both the absorbance at 260 nm and the radioactivity of the initial mixtures and the column effluent fractions were measured so that the disposition of each of the homopolymers could be known. It should be noted that mixing the two homopolymers resulted in as much as 30% hypochromic change when equivalent amounts of the two polynucleotides were used.

It can be seen that at high ionic strength, poly(U) is not retained by the cellulose whereas poly(A) is almost entirely adsorbed (Table I and Figure 1) and most of it is liberated when the salt content of the mobile phase is lowered (buffer K). Adsorption and elution are grossly similar whether done at room temperature (23°) or at refrigerator temperature (4°) (Figure 1).

The observed difference between cellulose binding of the radioactive and nonradioactive material in the poly(A) sample (Figure 1; Table I, line 4) was probably due to the presence of contaminating radioactive, non-poly(A) material or to poly(A) of inappropriate size classes in the commercial [^{3}H]poly(A) preparation. For our purposes the high specific activity [^{3}H]poly(A) was diluted with nonradioactive poly(A) so that absorbance at 260 nm could be used to trace the course of the chromatography. The chromatographic fidelity of a mixture prepared from the radioactive and nonradioactive poly(A) solutions was tested by fractionating a sample of it (2.162 A_{260} units, 124,680 cpm) using 430 mg of cellulose packed to 31-mm height in a column of 9-mm i.d. In this

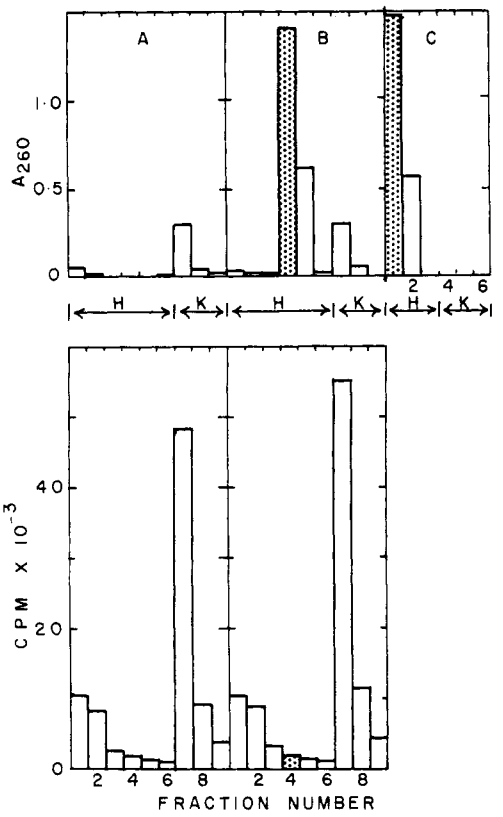

FIGURE 3: The effect of poly(C) on cellulose-bound poly(A). Two replicate samples of [^{3}H]poly(A) (0.478 A_{260} unit, 116,420 cpm) in 1 ml of buffer H were adsorbed to 60-mg cellulose columns (A and B). The columns were then washed with five 1-ml portions of buffer H followed by three 1-ml portions of buffer K. To column B was added poly(C) (2.090 A_{260} units) in the buffer H fraction 4 (stippled bar). The absorbance and radioactivity of each collected fraction are expressed by the bar heights in A and B. To column C only poly(C) (2.090 A_{260} units) was added at fraction 1. In this case buffer H was contained in fractions 1–3 and buffer K in fractions 4–6.

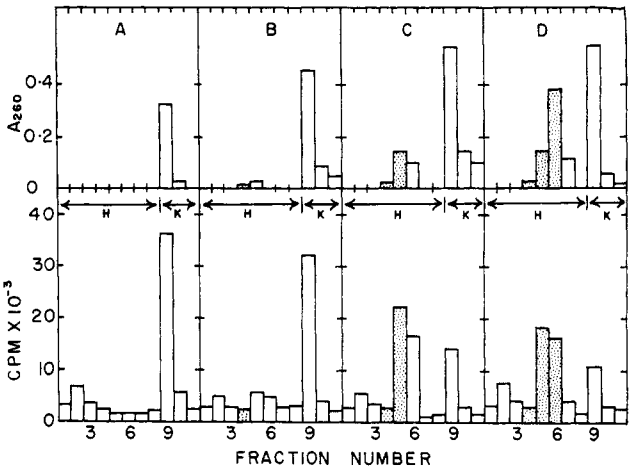

FIGURE 4: The binding of poly(U) to cellulose-bound poly(A). Four replicate samples of [^{3}H]poly(A) (0.488 A_{260} unit, 112,440 cpm) in 1 ml of buffer H were adsorbed to 60-mg cellulose columns. The columns were then washed with several 1-ml portions of buffer H followed by three 1-ml portions of buffer K. Poly(U) (0.452 A_{260} unit) was included in the buffer H in column additions at fraction 4 (column B), fractions 4 and 5 (column C), and fractions 4–6 (column D) (stippled bars). The absorbance and radioactivity of each collected fraction are expressed by the bar height. The regions indicated by H and K represent the fractions obtained during the passage of buffers H and K, respectively.

study 94% of the uv-absorbing material adhered to the cellulose and could be eluted at low ionic strength. Only 55% of the radioactive material behaved likewise, the rest passing through the column with buffer H. A portion of the fraction which bound was then rechromatographed on an identical cellulose column. In this repeat chromatogram virtually all of the uv-absorbing and radioactive material adhered to the cellulose and, in turn, eluted when the ionic strength was lowered. Thus, a major fraction of the commercial [^{3}H]poly(A) is sufficiently unlike the nonradioactive poly(A) to distinguish it from the latter by its cellulose adsorbability. This characteristic of our mixed poly(A) preparations accounts for the appearance of radioactivity in the column effluents during the passage of buffer H (Figures 2–4).

The slight effect of temperature on the radioactivity profile (Figure 1) may likewise be due to nonidentity of polynucleotide chain lengths between the radioactive and nonradioactive poly(A) preparations which were combined for these studies. Washing the column with buffer K liberates most of the adsorbed poly(A), leaving only a small amount still fixed to the cellulose (Figure 1, column c; Table I). In buffer H the poly(U)·poly(A) hybrids adsorb to cellulose poorly or not at all. A small but significant fraction of the ultraviolet-absorbing and radioactive material is retained by the cellulose even if the poly(U) component of the mixture is added in great excess (Table I, lines 5–7).

Elution of Poly(A) from Cellulose by Poly(U). Since poly(A)·poly(U) hybrids have little affinity for cellulose, attempts were made to remove bound poly(A) from cellulose at high ionic strength by including poly(U) in the elution buffer. An amount of poly(U) approximately equal to the amount of bound poly(A) did little to disengage poly(A) from the cellulose (Figure 2). In fact, the poly(U) was scavenged from solution as it passed through the column. As the proportion of poly(U) eluent was increased, an increasing proportion of the poly(A) appeared in the high-salt effluent and correspondingly a decreasing proportion remained to be flushed out by the subsequent buffer K wash. Even larger excesses of poly(U), however, failed to remove all the poly(A) from the column. Most of the residual polynucleotide was released when the salt concentration was subsequently lowered. Thus, there was a measurable amount of hybrid nucleic acid or non-hybrid poly(A) which resisted elution from cellulose by poly(U) but did not resist elution by the low ionic strength fluid. This resistant fraction was observed whether the poly(U) was mixed with the poly(A) before being added to the column or was added to the column which contained bound poly(A).

Effect of Poly(C) on Cellulose-Bound Poly(A). The results, shown in Figure 3, indicate that a large excess of poly(C) did not alter the elution profile of the [^{3}H]poly(A) and produced only such changes in the A_{260} profile as would be expected if the two polynucleotide species were noninteracting. The failure of poly(C) to remove poly(A) suggests that the poly(U) effect is attributable to its base complementarity to poly(A).

Retention of Poly(U) by Cellulose-Bound Poly(A). Cellulose-bound poly(A) is capable of retaining a large excess of poly(U) (Figure 4). The retention is most evident if the poly(U) is added stepwise to the column. Figure 4A depicts the binding of poly(A) in buffer H and the reversal of binding in buffer K. If an amount of poly(U) which is equivalent to the amount of cellulose-bound poly(A) is added to the column, neither poly(U) nor poly(A) appears in the effluent (Figure 4B). Successive additions of poly(U) (Figure 4B–D) release most but not all of the poly(A). Even when most of the poly(A) has

TABLE II: Selective Elution of Poly(A) from Cellulose.[a]

Added at fr 1	Poly(A)	Poly(A)	dDNA	dDNA	Poly(A) + dDNA	Poly(A) + dDNA	Poly(U)	Poly(A) + Poly(U)	dDNA + Poly(U)
Added at fr 4		Poly(U)		Poly(U)		Poly(U)			
Fr 1–3 A_{260}	0.004	0.000	0.011	0.015	0.036	0 051	0.432	0.407	0.484
cpm	3632	3656			4416	4768		11,680	
Fr 4–6 A_{260}	0.004	0.299	0.000	0.467	0.014	0.320	0.003	0.000	0.000
cpm	1568	3168			1576	2904		320	
Fr 7–11 A_{260}	0.136	0.094	0.097	0.093	0.202	0.184	0.009	0.014	0.095
cpm	4936	3128			4896	3,096		672	
Cellulose after fr 11, cpm	1080	802			755	915		10	
Cumulative recovery A_{260}	0.144	0.393	0.108	0.575	0.252	0.555	0.444	0.421	0.579
cpm	11,216	10,754			11,643	11,683		12,682	

[a] Poly(A): 8 μg (0.214 A_{260} unit)/0.4 ml in buffer H; 3.45×10^{-3} μCi, 13,198 cpm; poly(U): 20 μg (0.490 A_{260} unit)/0.4 ml in buffer H; dDNA: 8 μg (0.140 A_{260} unit)/0.4 ml in buffer H. Native calf thymus DNA was dissolved in buffer I at a concentration of 1 mg/ml, heated to 96° for 5 min, and rapidly chilled to 0°. The heat-denatured DNA was then diluted to 20 μg/ml in buffer H. On denaturation the DNA exhibited a 12% increase in absorbance at 260 nm. The combination solutions: poly(A) + dDNA (A_{260} = 0.353/0.4 ml), poly(A) + poly(U) (A_{260} = 0.450/0.4 ml). The columns used in this study consisted of Pasteur pipets in each of which was packed 43 mg of water-washed Sigmacell 38. The columns were then equilibrated against excess buffer H and allowed to drain. The indicated solutions (0.4 ml) were added to their respective columns and the effluent liquid was collected as fraction 1. Then 0.4 ml of buffer H was added to each column and the effluent liquid was designated fraction 2. The salt concentration of the solutions added to all columns for the first six fractions (0.4 ml at each fraction) was that of buffer H. Fraction 4 of columns 2, 4, and 6 contained poly(U) in the above designated amount. At each of the five fractions, 7–11, 0.4 ml of buffer K was added to each column and the effluent fractions were collected. The 260-nm absorbance of all fractions was measured and the radioactivity of fractions from the columns to which [^{3}H]poly(A) had been added was determined by drying a 50-μl sample of the effluent liquid on a 25-mm diameter glass fiber filter disk and counting it in 4 ml of scintillation fluid in a plastic 7-ml vial. The absorption and radioactivity of the original solution were likewise determined. After fraction 11 was collected the cellulose was extruded quantitatively onto a glass fiber disk, dried, suspended in 4 ml of scintillation fluid, and counted.

been removed from the cellulose by elution with excess poly(U) (Figure 4C,D), some remains bound and it retards the passage of further poly(U).

Selective Separation of Cellulose-Bound Poly(A) from Denatured DNA (dDNA). DNA, particularly single-stranded, binds to and elutes from cellulose under ionic conditions which promote the binding and elution of poly(A) and, if present in the extract, it contaminates the column fractions which contain poly(A)-linked RNA. Since at high ionic strength excess poly(U) elutes poly(A) from cellulose, it could serve as a selective eluting solute if it has no effect on the affinity of DNA for cellulose. This possibility was tested using poly(A) and calf thymus dDNA and the results are reported in Table II. The amount of poly(U) used in the elution was only about 2.3 times the amount of poly(A) and 3.5 times the amount of dDNA. As shown in Figure 2, larger amounts of poly(U) are more effective in eluting poly(A). However, the smaller amount of poly(U) was used so that its contribution to the uv absorbance would not obscure the absorbance due to the poly(A) and DNA.

The results show the characteristic binding and elution patterns of poly(A) with and without poly(U) (Table II, columns 1 and 2) and reveal the fraction of nonbinding radioactive poly(A) material. When dDNA was adsorbed to cellulose the amount of uv-absorbing material which was eluted with buffer K (columns 3 and 4, fractions 7–11) was essentially unaffected by prior passage of poly(U). When poly(A) and dDNA were added together, a slightly greater amount of absorbing and radioactive material emerged in buffer H (column 5, fractions 1 to 6) than when poly(A) and dDNA were chromatographed separately (columns 1 and 3). When a mixture of poly(A) and dDNA was adsorbed to cellulose, the passage of poly(U) (column 6) resulted in the elution of the same amount of absorbing and radioactive material as if poly(A) alone had been adsorbed (column 2).

If poly(A) or dDNA were mixed with poly(U) before being added to cellulose, the binding and elution of dDNA did not seem to be affected whereas the binding of poly(A) was sharply decreased (Table II, columns 8 and 9). The detailed uv elution profiles (not shown here) of poly(U), alone or with DNA (columns 4, 7, and 9), exhibited sharp absorbance peaks immediately following the addition of poly(U) to the columns; the recovery of poly(U) A_{260} units was virtually quantitative within a few fractions of the peak. When poly(A) was bound to cellulose the elution profile which followed the addition of poly(U) was much less sharp and the recovery of 260-nm-absorbing material was reduced. This tailing was probably due to interaction between the mobile and stationary polynucleotides which tended to delay passage of the otherwise mobile solute molecules (poly(U)). The reduction in total absorbance was due to the hypochromic effect. Thus, poly(U) interacted with cellulose-bound poly(A) but not with bound DNA. This result indicates an independent action of poly(U) in removing poly(A) from cellulose without affecting the resident status of DNA.

Discussion

The results reported here, together with those of other studies (Kitos *et al.*, 1972; Sullivan and Roberts, 1973; DeLarco and Guroff, 1973), indicate that unmodified cellulose or a contaminant of some preparations of cellulose has a high affinity for poly(A) and single-stranded DNA but virtually no affinity for either pyrimidine ribonucleotide homopoly-

mers, poly(A)-free heteropolymeric RNA, or double-stranded polynucleotides. The binding is influenced by both the pH and ionic strength of the system.

Why do some single-stranded polynucleotides bind to cellulose and others not? One possibility is that in high-salt solution the purine nucleotide homopolymers and single-stranded DNA exist in at least one molecular configuration which is improbable for pyrimidine nucleotide homopolymers and heteropolymeric RNA. In this exclusive form they would be complementary to and attracted by a nonrandom pattern of cellulose constituents, or of contaminants, possibly lignins (Sullivan and Roberts, 1973; DeLarco and Guroff, 1973), in association with the cellulose. The "cellulose"–polynucleotide association would depend upon weak, but cooperative interactions, possibly involving hydrophobic and/or hydrogen bonding. The proper configuration of the polynucleotide would be possible only under conditions which suppress or shield intrapolymeric electrostatic charges. Extended discussion of the molecular basis for binding must await clarification of the active constituents of cellulose preparations.

Poly(A) binds to cellulose at high ionic strength and elutes from it at low ionic strength. Exposed regions of cellulose-bound poly(A) anneal with poly(U). As shown in Table I and Figure 2, poly(U), which has no affinity for cellulose, can be retained by the poly(A)–cellulose complex. Excess poly(U) competes with cellulose for the poly(A) and displaces most of the poly(A) from the column. The remaining fraction can then be removed by a low-salt buffer. A 1:1 poly(U)·poly(A) hybrid, prepared in advance, passes virtually unrestrained through the cellulose bed. Thus, under these conditions the poly(U)·poly(A) hybrid appears to be more stable than the cellulose–poly(A) complex.

Since poly(A)-rich RNA makes up a very small part of the total cellular nucleic acids (Edmonds *et al.*, 1971) it is often necessary to separate it from DNA which occurs in the same extracts. It is possible to separate most of the poly(A)-containing RNA from DNA using cellulose adsorption followed by elution with poly(U) in high-salt buffer. Most double-stranded DNA passes directly through cellulose (Kitos *et al.*, 1972) but the small portion which binds, together with any DNA in the preparation, emerges from the column along with the poly(A)-containing RNA when the salt content of the mobile phase is lowered. However, poly(U) in buffer H elutes most of the poly(A)-containing constituents, leaving the DNA still bound to the cellulose. This method of separation is extremely effective but is not without problems since the poly(A)-linked RNA thus obtained is partly hybridized with poly(U) and exists in solution with a large excess of poly(U). Moreover, the poly(A) portion of the RNA in poly(U) hybrid form has no affinity for cellulose. Thus, further purification, if warranted, requires methods other than cellulose chromatography.

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