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Use of Benzoylated Cellulose Columns for the Isolation of Poly(adenylic acid) Containing RNA and Other Polynucleotides with Little Secondary Structure†

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ABSTRACT: Chromatography on benzoylated cellulose columns was found to be a simple and efficient procedure for the isolation of polynucleotides which contain extended nucleotide sequences free of secondary structure. Included among the polynucleotides which could be selectively bound to the benzoylated cellulose were poly(A), poly(U), poly(A)-containing RNA, formaldehyde-treated ribosomal RNA, and denatured DNA. Various conditions of adsorption and elution of polynucleotides were investigated; these indicated that the mechanism of binding is a stacking interaction between the polynucleotide bases and the benzoyl groups of the benzoylated cellulose. Using this procedure, milligram quantities of poly(A)-containing RNA were isolated from the cytoplasmic and nuclear fractions of Ehrlich ascites cells. These RNAs were analyzed by zonal centrifugation and nucleotide composition, using both ultraviolet adsorption and [^{32}P]orthophosphate labeling for a comparative analysis, and shown to be free from detectable contamination with ribosomal RNA.

Recently, a number of procedures have been developed for the isolation of poly(A)-containing RNAs from eukaryotes. These procedures can be classified into two main groups, depending upon the principle used for the isolation. One group utilizes for the isolation hydrogen bonding between the poly(A) moiety of the RNA and poly(U) or poly(dT) immobilized on glass filter membranes, cellulose, or Sepharose (Edmonds and Caramela, 1969; Kates, 1970; Adesnik *et al.,* 1972; Sheldon *et al.,* 1972). The second group of procedures is characterized by the firm binding at high salt concentrations of these RNAs to a variety of substances including methylated albumin (Asano, 1965; Ellem, 1966; Roberts and Quinlivan, 1969), polystyrene (Lim *et al.,* 1969; Lim and Canellakis, 1970), filter membranes (Lee *et al.,* 1971; Brawerman *et al.,* 1972), and cellulose (Sullivan and Roberts, 1971, 1973; Kitos *et al.,* 1972). The binding of poly(A)-containing RNA to filter membranes and cellulose appears to require the presence of polyaromatic lignins in the cellulose (Sullivan and Roberts, 1973; DeLarco and Guroff, 1973). This suggested a common aromatic ring stacking mechanism of binding for these latter procedures (Kitos, 1973), and prompted this investigation of benzoylated cellulose as a possible reagent with high capacity and flexibility for the isolation of poly(A)-containing RNA.

Experimental Section

Materials. Poly(A), poly(U), and poly(C) were purchased from Miles Laboratories. Poly(A)·poly(U) was prepared by mixing equal molar mixtures of poly(A) and poly(U), 50 μg/ml in 1 mM magnesium acetate; molar extinction coefficients at 260 nm of 9400 for poly(A) and 8900 for poly(U) were assumed. STE buffer was 0.3 M NaCl–0.1 M Tris·HCl (pH 7.0)–0.001 M EDTA; STE/10 buffer was STE diluted 1:10 with water. The ribosomal RNA (rRNA[1]) used in Table I was cytoplasmic RNA precipitated with 1.5 M NaCl and passed through a benzoylated cellulose column in 1 mM magnesium acetate to remove poly(A)-containing RNA. Heterogeneous cytoplasmic RNA (hcRNA) and heterogeneous nuclear RNA (hnRNA) are defined operationally in this paper as those RNAs from the corresponding cellular fractions which were synthesized in the presence of 0.04 μg/ml of actinomycin D (Perry, 1962; Roberts and Newman, 1966; Penman *et al.,* 1968) and could be precipitated with 1.5 M NaCl (for a review of heterogeneous RNAs see Darnell, 1968 and Darnell *et al.,* 1973); the hcRNA and hnRNA in Table I were poly(A)-containing hRNAs isolated from benzoylated cellulose columns and rerun to determine binding capacity. Formaldehyde-treated rRNA was prepared by dissolving rRNA in STE/10 (100 μg/ml), making the solution 1 M in formaldehyde and heating the solution at 60° for 10 min; the RNA was precipitated by adjusting the NaCl concentration to 0.1 M, mixing the solution with an equal volume of isopropyl alcohol, and placing the solution at −20° overnight. Denatured DNA was prepared by dissolving calf thymus DNA (Calbiochem) in distilled water, heating the solution to 90° followed by quick cooling, and adjusting the solution to 1 mM in magnesium acetate. Mengovirus RNA was prepared as before (Sullivan and Roberts, 1973).

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[1] Abbreviations used are: rRNA, ribosomal RNA; hcRNA, heterogeneous cytoplasmic RNA; hnRNA, heterogeneous nuclear RNA; nRNA, nuclear RNA; cRNA, cytoplasmic RNA; STE buffer, 0.3 M NaCl-0.1 M Tris-HCl (pH 7.0)-0.001 M EDTA; STE/10 buffer, STE diluted 1:10 with water.

TABLE I: Binding Capacity of Benzoylated Cellulose for Various Polynucleotides.[a]

Polynucleotide	Conditions for Binding	A_{260} Units Bound/ml of Benzoylated Cellulose
Poly(A)	STE	97
Poly(A)	STE/10	14
Poly(A)	STE/10 (0°)	4
Poly(A)	MA[b]	20
Poly(U)	STE	77
Poly(U)	STE/10	0.8
Poly(U)	0.1 mM spermidine	60
Poly(U)	0.1 mM spermidine (4°)	0.2
Poly(U)	MA	30
Poly(A)·poly(U)	MA	25%[c]
Poly(C)	STE	0.17
Poly(C)	MA	0.03
rRNA	STE (100 absorbance units added)	2
rRNA	MA (400 absorbance units added)	0.4
rRNA	MA (20 absorbance units added)	0.08
rRNA	MA (2 absorbance units added)	0.02
rRNA (Formaldehyde-treated)	MA	12
hcRNA	MA	3.0
hnRNA	MA	2.9
Denatured DNA	MA	7.6
Mengovirus RNA	MA (4 absorbance units added)	0.35

[a] Capacities were determined by passing at least a 100% excess of polynucleotide through the column. Chromatography was at 23° unless indicated otherwise; 1 ml of benzoylated cellulose is equivalent to approximately 0.4 g. [b] MA is 1 mM magnesium acetate. [c] This fraction of poly(A)·poly(U) bound to the column independent of the amount added (2, 10, and 50 absorbance units were tested).

Benzoylated cellulose was prepared according to the procedure of Gillam *et al.*, 1967.

Preparation of RNAs. The propagation of ascites cells, fractionation of cells into nuclear and cytoplasmic components, and extraction of RNA at pH 9.5 have all been previously described (Roberts *et al.*, 1966). The initial alcohol precipitate of nRNA was dissolved in 4 ml of sterile water (for each 1.5 ml or original ascites cells), and to this solution was added 0.2 ml of 3 M sodium acetate buffer (pH 5.3), 0.04 ml of 0.1 M magnesium acetate, 0.04 ml of 10 mg/ml dextran sulfate (Sigma), and 0.04 ml of 1 mg/ml deoxyribonuclease I (Worthington, premium grade). This solution was incubated at 37° for 10 min and the RNA precipitated by the addition of 2 volumes of ethanol. The nRNA was purified further by dissolving in 1 ml of water, adding 0.05 ml of the acetate buffer and 1 ml of 3 M NaCl, and allowing the RNA to precipitate overnight at 4°. The cytoplasmic RNA was reprecipitated with ethanol and, in the case of ^{32}P-labeled RNA, reprecipitated again with 1.5 M NaCl. The labeling of ascites cells with [^{32}P]orthophosphate was as described (Sullivan and Roberts, 1973) except it was carried out for 2 hr.

Chromatography on Benzoylated Cellulose Columns. Columns were packed with benzoylated cellulose (8 ml) under low air pressure and washed with 50% ethanol until the absorbance of the effluent at 260 nm read 0.02 or less. Polynucleotides were dissolved in buffer at a concentration of 20–200 μg/ml and placed in a reservoir. This solution was run through the column at 23° with a flow rate of approximately 1 ml/min. The column was then washed with elution buffer until the absorbance of the effluent was 0, and the bound polynucleotide was eluted by the repeated additions of 5-ml portions of ethanol–0.1 mM Na_4EDTA (1:1). Polynucleotides eluted primarily in the second fraction with a small amount of material also present in the third fraction. Fractions containing eluted RNA were made 0.15 M in sodium acetate buffer and the RNA precipitated by the addition of 0.5 volume of isopropyl alcohol. This RNA was dissolved in 10 ml of 1 mM magnesium acetate and passed through a second benzoylated cellulose column to remove a small amount of contaminating rRNA. The bound RNA from the second column was eluted as before and alcohol precipitated to prepare samples for sedimentation analysis; for nucleotide composition analysis, RNA in the eluted fractions was precipitated by adjusting the solutions to 5% in trichloroacetic acid. Experimental details for these analyses have been described (Roberts, 1965).

Results

Binding Specificity of Benzoylated Cellulose Columns. In the presence of STE buffer, unmodified cellulose columns have been shown to have a much greater capacity for binding poly(A) and poly(A)-containing RNAs than binding poly(U) or rRNA (Sullivan and Roberts, 1973; Kitos and Amos, 1973). Benzoylation of Whatman CF_{12} cellulose, a cellulose with a low lignin content (Sullivan and Roberts, 1973), increased dramatically the capacity of the cellulose to bind all types of polynucleotides (Table I). The benzoylated cellulose maintained a strong preference for binding poly(A) compared with rRNA, but it also showed a very high capacity for binding poly(U). Dilution of the STE buffer tenfold reduced the binding capacity of the benzoylated cellulose for all polynucleotides and the preferential binding of poly(A) compared with poly(U) was again observed (Table I). Lowering the temperature of the

poly(A) solution to 0° caused a hypochromic shift in adsorption due to an increase in base stacking within the poly(A) molecules (Leng and Felsenfeld, 1966); this was accompanied by a decrease in the binding of poly(A) to the column.

Very low concentrations of magnesium ions, spermine, or spermidine were found to be sufficient to promote efficient binding of polynucleotides to benzoylated cellulose. Ionic conditions similar to these are known to promote base stacking within poly(U) molecules (Lipsett, 1960; Szer, 1966), and presumably also could encourage a stacking interaction between bases in the polynucleotide and benzoyl groups on the column. In 0.1 mM spermidine, poly(U) showed a dramatic hypochromic effect upon lowering the temperature from 23 to 4°. This temperature drop also eliminated the ability of poly(U) to bind to benzoylated cellulose (Table I). Furthermore, poly(U) bound at 23° could be eluted from the benzoylated cellulose with cold 0.1 mM spermidine after cooling the column for several hours to 4°. Poly(C), in contrast to poly(U), would be expected to contain a significant amount of intramolecular base stacking in STE or 1 mM magnesium acetate (Fasman *et al.*, 1964; Brahms *et al.*, 1967), and in these buffers this polynucleotide bound very poorly to the benzoylated cellulose.

The ascites cell RNAs analyzed in this paper were fractionated on benzoylated cellulose columns using 1 mM magnesium acetate as the elution buffer. At this magnesium ion concentration, the columns efficiently bound poly(A), poly(U), and poly(A)-containing hcRNA and hnRNA, whereas rRNA bound very poorly (Table I). Over 85% of the purified poly(A)-containing hcRNA and hnRNA bound to the column upon rechromatography. Recovery of this RNA from the column was routinely greater than 80%; recovery could be made almost quantitative by lengthening the elution time and increasing the volume of 50% ethanol used for elution. The addition of between 2 and 400 A_{260} units of rRNA to the column resulted in between 1 and 0.1% of the added rRNA binding to the benzoylated cellulose; the fraction of bound rRNA was constant with repeated passages of a single sample of rRNA through the column. Benzoylated cellulose columns were reused more than ten times without seriously affecting their efficiency for isolating poly(A)-containing RNA. However, with continued use the capacity of the columns for binding all polynucleotides gradually decreased, presumably because of the slow hydrolysis and release of benzoyl groups from the cellulose.

Double-stranded polynucleotides bound poorly to benzoylated cellulose, as they did to unmodified cellulose (Sullivan and Roberts, 1973; Kitos and Amos, 1973), presumably because the hydrogen bonding stabilizes the intramolecular base stacking and allows insufficient stacking interaction between the bases and the benzoyl groups. A constant 25% of poly(A)·poly(U) was found to bind to benzoylated cellulose independently of the amount applied to the column (up to 50 A_{260} units); this probably represents the fraction of poly(A)·poly(U) molecules which contain single-stranded tails sufficiently long to promote binding. Formaldehyde treatment of polynucleotides is known to destroy hydrogen-bonded secondary structure while permitting a degree of single-stranded base stacking (Fasman *et al.*, 1964; Boedtker, 1967). rRNA treated in this manner bound effectively to the column as did denatured DNA (Table I), indicating that polynucleotide binding to benzoylated cellulose requires lack of secondary structure and is not dependent upon poly(A) or poly(U) regions within the polynucleotide. Mengovirus RNA, which is known to contain poly(A) stretches considerably shorter than those found in hcRNA (Miller and Plagemann, 1972; Sullivan and Roberts, 1973), did not bind as efficiently as hcRNA to the benzoylated cellulose. This suggests that the length of the unstructured region of a polynucleotide is important in determining its capacity to bind to benzoylated cellulose.

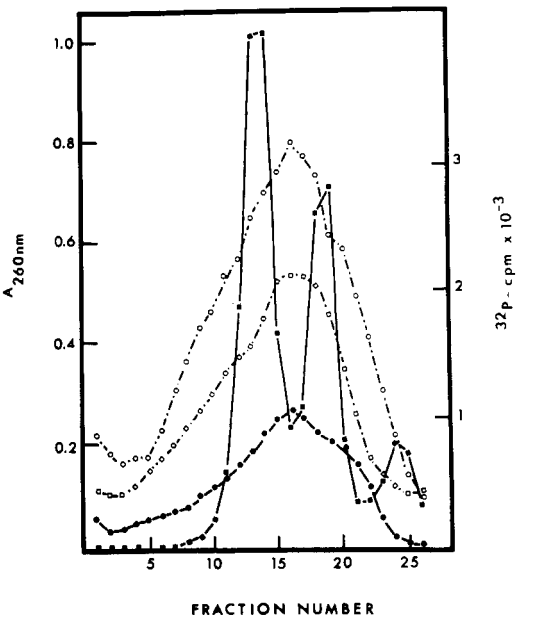

FIGURE 1: Zonal centrifugation of cRNA. Each RNA sample was dissolved in 1.0 ml of gradient buffer (0.05 M NaCl–0.01 M sodium acetate buffer (pH 5.3)–0.001 M magnesium acetate), layered onto 26 ml of a 5–20% sucrose gradient in gradient buffer, and centrifuged in a SW 25.1 Spinco rotor at 21,000 rpm for 15 hr at 0°. Fractions of 1.0 ml were collected and 0.2 ml of each was plated and counted for radioactivity measurements; top of gradient was fraction 26, 18S RNA was fraction 19, 28S RNA was fraction 14. (■) Absorbance at 260 nm of unbound cytoplasmic RNA from 0.06 g of ascites cells. (●) Absorbance of bound hcRNA from 3 g of ascites cells. (○) Radioactivity of bound hcRNA from 1.4 g of ascites cells labeled for 2 hr with [^{32}P]orthophosphate. (□) Radioactivity of bound hcRNA from 1.4 g of ascites cells labeled with [^{32}P]orthophosphate for 2 hr in the presence of 0.04 μg/ml of actinomycin D.

Analysis of hcRNA and hnRNA Isolated Using Benzoylated Cellulose Columns. Chromatography on benzoylated cellulose resulted in approximately 65% of the total hcRNA and 55% of the total hnRNA from ascites cells binding to the columns (see Experimental Section for a definition of hRNA). Hybridization of the bound RNA with [^{3}H]poly(U) (Sullivan and Roberts, 1973) revealed that the bound fractions contained over 95% of the poly(A) regions present in both the cytoplasmic and nuclear RNAs. These values are similar to those obtained using unmodified cellulose columns (Sullivan and Roberts, 1973), and showed that benzoylated cellulose is an efficient adsorbant of poly(A)-containing hcRNA and hnRNA.

The bound fractions of hcRNA and hnRNA were analyzed by zonal centrifugation and nucleotide composition analysis as a measure of the degree of contamination of these preparations with rRNA. Also, the capacity and simplicity of the benzoylated cellulose column procedure permitted the isolation of relatively large amounts of hcRNA and hnRNA, enabling us to compare for the first time sedimentation profiles and nucleotide compositions using both absorbance and ^{32}P-labeling as means of detection.

The sedimentation profiles of various cytoplasmic RNA fractions are presented in Figure 1. Absorbancy measurements showed the usual large peaks of 28S and 18S rRNA and a small 4S peak of tRNA in the unbound fraction; the RNA which bound to the column was heterogeneous in distribution with a peak at approximately 23 S. The bound RNA labeled in

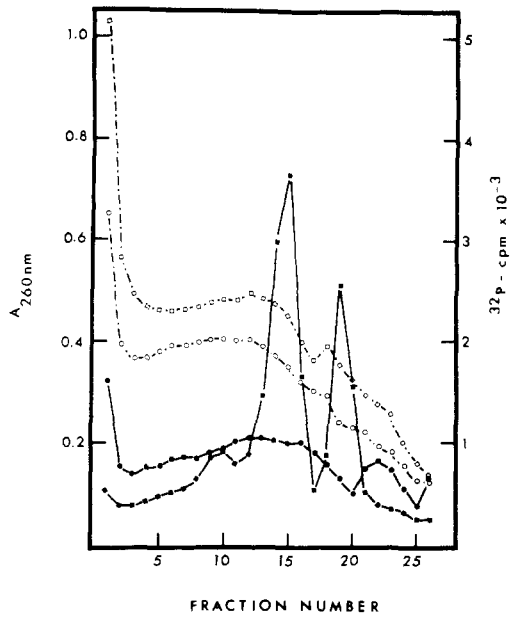

FIGURE 2: Zonal centrifugation of nRNA. Samples were centrifuged and analyzed as in Figure 1 except that centrifugation was at 19,000 rpm for 15 hr at 0°. The top of the gradient was fraction 26, 18S RNA was fraction 19, 28S RNA was fraction 15. (■) Absorbance at 260 nm of unbound nRNA from 1 g of ascites cells. (●) Absorbance of bound hnRNA from 4 g of ascites cells. (□) Radioactivity of bound hnRNA from 1.4 g of ascites cells labeled for 2 hr with [^{32}P]orthophosphate in the presence of 0.04 μg/ml of actinomycin D. (□) Radioactivity of unbound hnRNA from the same actinomycin D treated cells.

the absence of actinomycin gave a sedimentation profile similar to that detected with absorbancy, indicating similar specific activities among the various sedimentation classes of bound hcRNA and suggesting that there are no large differences in the rate of turnover of these classes of RNA within the cell. The bound RNA labeled in the presence of 0.04 μg/ml of actinomycin D showed a sedimentation profile similar to the two above; this is further evidence that this concentration of actinomycin does not affect significantly the synthesis of hcRNA. rRNA was not detected in the profiles of any of the bound hcRNA fractions.

Zonal centrifugation of the nuclear RNA fractions (Figure 2) revealed in the unbound fraction a small peak of 45S ribosomal precursor RNA followed by larger peaks of 28S and 18S rRNA. Absorbancy measurements on the bound hnRNA showed a broad band of rapidly sedimenting RNA together with another distinct peak at fraction 22; this latter peak had little radioactivity associated with it, and it is not known whether this material represents slowly labeled RNA, or DNA which had survived deoxyribonuclease treatment and NaCl precipitation. No rRNA could be detected in the bound hnRNA.. Both bound and unbound ^{32}P-labeled hnRNAs had similar sedimentation profiles, which did not differ significantly from the absorbancy profile of bound hnRNA.

The nucleotide compositions of various fractions of nuclear and cytoplasmic RNAs are recorded in Table II. Absorbancy measurements showed the nucleotide compositions of bound hcRNA and hnRNA to resemble each other closely and to be very different from the corresponding unbound RNA fractions. The bound hcRNA and hnRNA fractions were assayed for poly(A) content by hybridization to [^{3}H]poly(U), and gave results very similar to those observed for hcRNA and hnRNA bound to unmodified cellulose (Sullivan and Roberts, 1973); all sedimentation classes of these RNAs contained poly(A) and the fraction of total nucleotides present as poly(A) decreased with increasing sedimentation rate of the RNAs. If the current model for poly(A)-containing hcRNA and hnRNA is correct, these RNA molecules would each contain a single poly(A) region of similar size at the 3′ terminus (Darnell *et al.*, 1973); this would explain the lower per cent of adenylic acid in hnRNA compared with the more slowly sedimenting, and presumably much smaller, hcRNA molecules. The lower per cent of adenylic acid observed in the more rapidly sedimenting fractions of ^{32}P-labeled bound hcRNA and hnRNA is also consistent with the above model. The ^{32}P-nucleotide compositions of the bound and unbound (act. D) hcRNA fractions were generally similar to each other and to the absorbancy nucleotide composition of total bound hcRNA. The ^{32}P-nucleotide composition of bound hnRNA resembled the absorbancy composition of hnRNA and the compositions of hcRNA except that the ^{32}P-labeled hnRNA showed a significantly higher per cent of uridylic acid. This unusually high uridylic acid content of ^{32}P-labeled hnRNA has been consistently observed over many years (Roberts and Newman, 1966; Roberts and Quinlivan, 1969; Sullivan and Roberts, 1973); the absence of high uridylic acid in the absorbancy nucleotide composition of bound hnRNA suggests that the high ^{32}P-nucleotide composition value is an artifact arising from unequal labeling of the nuclear nucleoside triphosphate pool or a ^{32}P-labeled contaminant migrating during electrophoresis with uridylic acid. The unbound hnRNA resembled bound hnRNA in its ^{32}P-nucleotide compositions except for its lower per cent of adenylic acid, probably a result of the unbound fraction lacking poly(A). This similarity between bound and unbound hnRNA, both in nucleotide composition and sedimentation profile (Figure 2), suggests that unbound hnRNA may represent that portion of a common hnRNA pool which had not been polyadenylated and/or it was derived from bound hnRNA by cleavage of the poly(A) moiety.

Discussion

Studies on the conformation of polynucleotides in aqueous solution have yielded information useful in considering the possible mechanism of binding of polynucleotides to benzoylated cellulose. At neutral pH poly(A) (Brahms *et al.*, 1965; Stevens and Rosenfeld, 1966; Eisenberg and Felsenfeld, 1967) and poly(C) (Fasman *et al.*, 1964; Brahms *et al.*, 1967) have been shown to exist as single-strand helical structures stabilized by base stacking and containing little, if any, hydrogen bonding. The extent of the base stacking in these molecules varies with temperature and ionic strength, increasing with increasing salt concentration or decreasing temperature. In contrast, poly(U) normally exists as a highly extended random coil containing little base-stacked structure (Inners and Felsenfeld, 1970). A hypochromic shift, indicating the formation of an ordered base-stacked form, does occur with poly(U) at NaCl concentrations greater than 0.5 M (Simpkins and Richards, 1967), at low magnesium ion concentrations (Lipsett, 1960), and at even lower concentrations of spermine or spermidine (Szer, 1966). Since these are approximately the conditions which promote the binding of poly(U) to benzoylated cellulose, it suggests that the binding mechanism involves a stacking interaction between the polynucleotide bases and benzoyl groups on the cellulose. This view is strengthened by the observation that conditions which increase intramolecular base stacking (poly(A) in STE/10 at 0°; poly(U) in 0.1 mM spermidine at 4°; poly(A)·poly(U) in 1 mM magnesium acetate) reduce or eliminate the capacity of the polynucleotide to bind to the column (Table I), presumably because the stacked bases are no longer available for in-

TABLE II: Nucleotide Compositions of Nuclear and Cytoplasmic RNAs.

Fraction Analyzed	Method of Analysis	Mole %			
		C	A	G	U
hcRNA (bound)	Absorbance	20.7	31.7	22.5	25.2
cRNA (unbound)		29.4	17.9	34.0	18.8
hnRNA (bound)		23.7	28.4	23.2	24.7
nRNA (unbound)		30.0	17.4	33.1	19.4
hcRNA (bound, 1–6)[a]	^{32}P	23.6	27.5	22.3	26.6
hcRNA (bound, 7–12)		23.6	27.4	22.7	26.3
hcRNA (bound, 13–18)		24.0	28.3	22.1	25.7
hcRNA (bound, 19–24)		22.8	30.9	20.1	26.2
hcRNA (bound, act. D, 1–6)[b]	^{32}P	24.8	28.0	21.5	25.6
hcRNA (bound, act. D, 7–12)		24.5	28.9	21.1	25.5
hcRNA (bound, act. D, 13–18)		24.9	29.5	20.9	24.7
hcRNA (bound, act. D, 19–24)		23.4	32.9	19.9	23.8
hnRNA (bound act. D, 1–6)	^{32}P	22.6	26.6	21.9	28.9
hnRNA (bound, act. D, 7–12)		22.2	26.3	22.8	28.7
hnRNA (bound, act. D, 13–18)		22.9	27.3	22.1	27.7
hnRNA (bound, act. D, 19–24)		21.7	28.8	21.1	28.4
hnRNA (unbound, act. D, 1–6)	^{32}P	23.0	24.2	22.0	30.8
hnRNA (unbound, act. D, 7–12)		23.6	23.3	22.6	30.5
hnRNA (unbound, act. D, 13–18)		24.5	21.8	24.0	29.7
hnRNA (unbound, act. D, 19–24)		22.9	22.1	24.0	30.9

[a] The designation (bound, 1–6) means that fractions 1–6 from the zonal centrifugation of bound hcRNA (Figure 1) were combined and analyzed for nucleotide composition. [b] The (bound, act. D, 1–6) means that the fractions analyzed were from bound hcRNA isolated from cells labeled in the presence of 0.04 μg/ml of actinomycin D.

teraction with the benzoylated cellulose. The adsorption of formaldehyde-treated rRNA and denatured DNA to the column (Table I) also is consistent with a stacking mechanism of binding.

The requirements for binding, therefore, appear to be that the polynucleotide must contain extended regions of unstacked bases, and that the conditions of cation concentration and temperature used in the chromatography must be such that a stacking interaction with the benzoylated cellulose is encouraged but base stacking within the polynucleotide is not raised above a critical level. For example, the adsorption of poly(U) to the column decreased markedly following a shift in the elution buffer from STE to STE/10 because the ionic strength was too low to permit efficient interaction of the uracil bases with the benzoylated cellulose; in contrast, lowering the temperature of a 0.1 mM spermidine solution from 23 to 4° increased stacking forces to the point that poly(U) was in the completely ordered form with its bases unavailable for binding. Also, another consideration in the binding reaction is that purines interact with benzoylated cellulose more effectively than pyrimidines. The partially base-stacked form of poly(A) bound efficiently to the columns under a variety of conditions whereas the partially base-stacked form of poly(C) did not, under any conditions tested (Table I). Poly(U) did bind well to the columns, but only in the random coil form and only under conditions which strongly promoted a stacking interaction.

Chromatography on benzoylated cellulose columns proved to be a simple and efficient method for the large-scale isolation of poly(A)-containing RNAs from eukaryotes. The RNA obtained using this procedure was free from detectable rRNA, was apparently undegraded, and was similar to the hRNAs isolated from ascites cells using other methods (see Sullivan and Roberts, 1973). Poly(A)-containing RNA obtained in this manner should be useful for a variety of biochemical experiments; for example, the bound hcRNA has been shown to stimulate an *in vitro* protein synthesizing system from ascites cells (Sharma, 1973). Isolation of poly(A)-containing RNAs on benzoylated cellulose appears similar in mechanism to their isolation on filter membranes (Lee *et al.*, 1971; Brawerman *et al.*, 1972) and unmodified cellulose (Kitos *et al.*, 1972; Sullivan and Roberts, 1973). However, benzoylation increases the reactivity of the cellulose, resulting in a greatly increased capacity of the benzoylated cellulose to bind poly(A), together with an ability to bind polynucleotides, such as poly(U), which do not bind to the less reactive adsorbants. In comparison with methods utilizing the binding of poly(A)-containing RNAs to poly(U)- or or poly(dT)-cellulose, Sepharose, or glass filter membranes, benzoylated cellulose columns have the advantage of avoiding any possibility of solubilized poly(U) or poly(dT) contaminating the RNA sample. These columns have the disadvantage that they are not as efficient at binding RNAs containing short regions of poly(A). Mengovirus RNA does not bind to benzoylated cellulose as effectively as hcRNA or hnRNA (Table I), and preliminary experiments have indicated that hemoglobin 9S mRNA, which also contains a short poly(A) sequence (Morrison *et al.*, 1973), does not bind efficiently to benzoylated cellulose in 1 mM magnesium acetate but can be isolated by several passages of the bound fraction of rabbit reticulocyte RNA through columns using STE as the adsorption buffer.

Benzoylated cellulose columns should prove useful for polynucleotide fractionation other than the isolation of poly(A)-containing RNAs. Variations in the elution buffer, elution temperature, and column size should give this procedure wide flexibility for isolating polynucleotides which contain extended regions with little secondary structure. For example, prelimi-

nary experiments in this laboratory indicate that chromatography of cytoplasmic RNA in 1 mM magnesium acetate to remove poly(A)-containing hcRNA, followed by chromatography of the effluent in STE buffer, leads to the isolation of a fraction of hcRNA which contains little, if any, poly(A). Other potential uses of this procedure include the separation of single- and double-stranded polynucleotides, and the fractionation of poly(A) or poly(U) into various molecular weight classes.

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