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Note

Purification of polyadenylated mRNA on three oligo(dT)-substituted gels: a comparative study

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Eukaryotic mRNA contains a covalently attached 3′ terminal poly(A) segment which provides the basis for affinity chromatographic techniques employed in the isolation of mRNA. Thus, poly(A)$^+$–mRNA can be separated from other cellular RNA by affinity chromatography on poly(U)–agarose[1] or oligo(dT)–cellulose[2,3] which is the most widely used support in affinity chromatography. Typically, a sodium chloride solution of RNA is passed over these affinity supports to allow the poly(A)–oligo(dT) base pairing interaction[4,5]. Poly(A) minus RNA passes through the column whereas the adsorbed RNA is eluted upon decreasing the ionic strength. However, oligo(dT)–cellulose has disadvantages related to the non-specific adsorption of RNA on the cellulose matrix[6]: poly(A) minus RNA may be adsorbed on oligo(dT)–cellulose by interactions with the aromatic moiety of lignine present in cellulose preparations[7,8]. Poly(U)–agarose also requires poly(A)$^+$–mRNA binding conditions similar to those of oligo(dT)–cellulose. The interaction of a poly(U) ligand with a poly(A)$^+$–mRNA segment is usually very strong, due to the size of the immobilized poly(U) chain, and thus requires stronger elution conditions. Non-specific adsorption of poly(A) minus RNA to poly(U)–agarose is minimal.

The purpose of this study was the synthesis of different affinity adsorbents which would specificially retain poly(A)$^+$–mRNA and from which the latter could be desorbed using moderate elution conditions, thus avoiding the drawbacks of the commonly used affinity adsorbents oligo(dT)–cellulose and poly(U)–agarose.

MATERIALS AND METHODS

Chemicals

5′-dTMP and poly(A) (MW 100,000) were purchased from Boehringer (Mannheim, G.F.R.), dicyclohexylcarbodiimide, pure pyrimidine and pure dimethylformamide from Aldrich Europe (Beerse, Belgium). Ultrogel A4 (4% agarose gel), Trisacryl [poly(N-acryloyl-2-amino-2-(hydroxymethyl)-1,3-propanediol)] and Acriflavin-Ultrogel A4R Type I were from Réactifs IBF (Villeneuve la Garenne, France), cellulose CF-11 from Whatman (Springfield, Great Britain), oligo(dT)–cellulose (Lot 81

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F-7260), acrylamide and N,N'-methylenebisacrylamide for electrophoresis from Sigma (St. Louis, MO, U.S.A.). Cell-free protein synthesizing system, [^{35}S]methionine, [^{3}H]poly(C) and EN^{3}HANCE autoradiography enhancer solution were purchased from NEN (Boston, MA, U.S.A.). Oviduct RNA was generously supplied by Dr. M. Lemeur.

Oligo(dT)–Ultrogel and oligo(dT)–Trisacryl synthesis

The synthesis of oligo(dT) was carried out as described by Tener *et al.*[9] with minor modifications. Briefly, the pyridinium salt of 5'-dTMP (2.7 mol) was dissolved in dimethylformamide (50 ml) containing 5 mmol of dicyclohexylcarbodiimide. The formation of oligomers was monitored by chromatography on Acriflavin-Ultrogel A4R Type I[10]. Oligo(dT) was immobilized on dried Ultrogel A4 or Trisacryl using dicyclohexylcarbodiimide as described for the synthesis of oligo(dT)–cellulose[11]. After coupling, the substituted gel was washed successively with dimethylformamide, ethanol, water and finally with 1 *M* sodium chloride solution. The yield of the coupling reaction was higher than 40%.

The amount of oligo(dT) immobilized was determined after total hydrolysis of the gels. The concentration of released 5'-dTMP was measured from the absorbance at 254 nm.

Cell-free protein synthesizing assays

Cell-free mRNA translation, using the mRNA-dependent protein-synthesizing system, derived from rabbit reticulocyte lysate, was carried out as previously described[12]. Purified mRNA (0.1–0.5 μg) was mixed with the cell-free system (10 μl) in the presence of [^{35}S]methionine as radioactive marker. The solution was incubated at 37°C for 60 min and then the reaction was stopped by cooling the solution in an ice-bath. An aliquot (2 μl) was adsorbed on a Whatman paper, washed with a 10% trichloroacetic acid solution at 4°C and heated at 90°C for 20 min in 5% trichloroacetic acid; the paper was then dried with ethanol and diethyl ether. The radioactivity was measured in a liquid scintillation spectrometer.

For qualitative analysis, the translated products from oviduct poly(A)$^{+}$–mRNA were analysed by electrophoresis in 10–20% gradient polyacrylamide gels containing 0.1% sodium dodecyl sulphate (SDS) as described by Laemmli[13]. The dried gel was exposed to Kodak film overnight.

After migration, the gel was transferred to an EN^{3}HANCE solution and the proteins were observed fluorimetrically.

RESULTS AND DISCUSSION

Specificity and adsorption of oligo(dT)–gels

The characteristics of the oligo(dT) gels are summarized in Table I and Fig. 1. The latter shows that the adsorption capacity of the three oligo(dT) matrices, for synthetic poly(A), is dependent on the salt concentration which favour base pairing association. Nevertheless, the adsorption capacity of oligo(dT)–cellulose is altered by a high non-specific adsorption due to the matrix itself (Table I, Fig. 1B). This non-specific adsorption results from some hydrophobic or aromatic groups present in the cellulose preparation, as described elsewhere[8]. On the contrary, the adsorption ca-

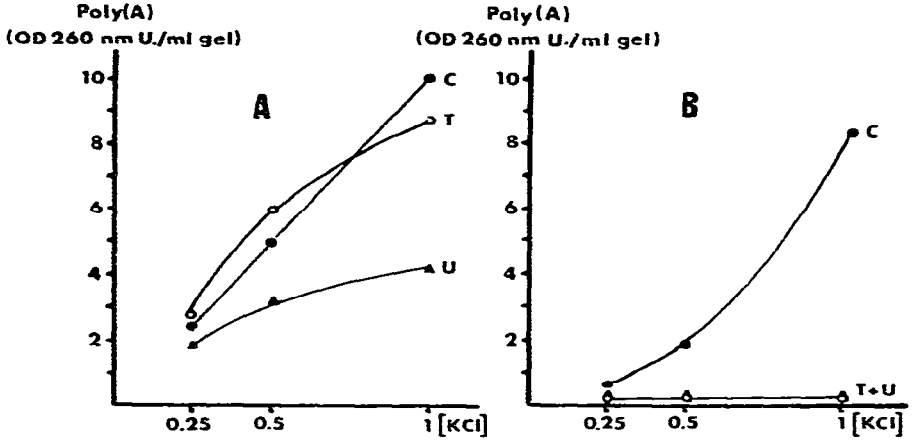

Fig. 1. Poly(A) adsorption on oligo(dT)-substituted gels (A) and on native gels (B) as a function of KCl concentration. 2 ml of poly(A) solution (1 mg/ml) in 10 m*M* Tris–HCl buffer pH 7.5 containing potassium chloride at different concentrations was applied to a 1-ml column. The solution was recycled five times and the column extensively washed with the adsorption buffer. The adsorbed poly(A) was then eluted using potassium chloride-free buffer. The amount of adsorbed and eluted polynucleotide was determined spectrophotometrically at 260 nm. U = Ultrogel; T = Trisacryl; C = cellulose.

pacity of oligo(dT)–Ultrogel and oligo(dT)–Trisacryl for poly(A) increases proportionally with the ionic strength (Table I, Fig. 1A); the corresponding unsubstituted gels dot not adsorb poly(A) molecules, even at high salt concentration, as a result of the hydrophilicity of the matrices. Morever, oligo(dT) matrices present a high specificity, since adsorption of poly(C) molecules is negligible even at high salt concentration (data not shown).

TABLE I

POLY(A) BINDING ASSAYS OF OLIGO(dT)-SUBSTITUTED GELS

All experiments were performed as indicated in Fig. 1: poly(A) solution was adsorbed in 10 m*M* Tris–HCl buffer pH 7.5 containing either 0.25 *M* or 0.50 *M* potassium chloride.

	Ultrogel		*Trisacryl*		*Cellulose*	
	Oligo(dT)-substituted	*Unsubstituted*	*Oligo(dT)-substituted*	*Unsubstituted*	*Oligo(dT)-substituted*	*Unsubstituted*
Oligo(dT) (mg immobilized per ml of gel)	5	–	8.8	–	13	–
0.25 M KCl						
Poly(A) (O.D. units at 260 nm per ml of gel)	1.99	0.0	2.61	0.0	2.3	0.47
Adsorption efficiency [O.D. units of poly(A) per mg of oligo(dT)]	0.398	–	0.296	–	0.176	–
0.5 M KCl						
Poly(A) (O.D. units at 260 nm per ml of gel)	3.27	0.0	5.89	0.0	4.88	1.98
Adsorption efficiency [O.D. units of poly(A) per mg of oligo(dT)]	0.654	–	0.669	–	0.375	–

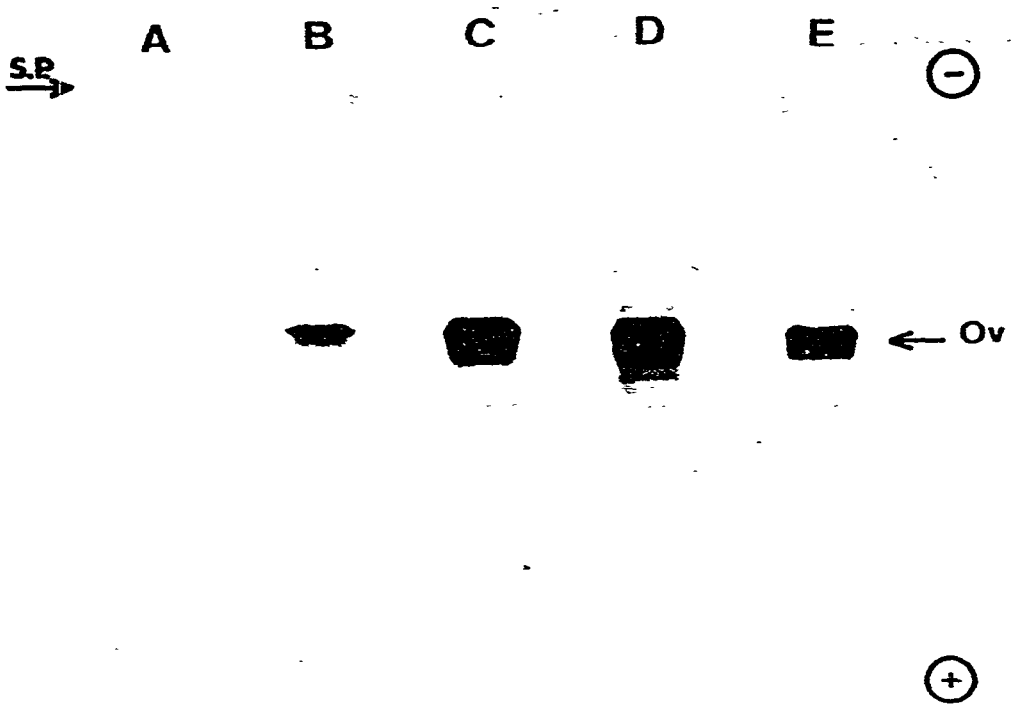

Fig. 2. Polyacrylamide gel electrophoresis of poly(A)$^+$-mRNA translation products (autoradiography). The amount of biological material (translation mixture) submitted to electrophoresis was of 20 μl. S.P. = Starting point of the electrophoresis. For details, see Materials and Methods. A = Control without poly(A)$^+$-mRNA; B = translation products from crude RNA; C, D, E = translation products from poly(A)$^+$-mRNA collected respectively from oligo(dT)-Ultrogel, oligo(dT)-Trisacryl and oligo(dT)-cellulose.

TABLE II

AFFINITY CHROMATOGRAPHY OF POLY(A)$^+$-mRNA FROM OVIDUCT EXTRACT ON OLIGO(dT)-SUBSTITUTED GELS

Total RNA from chicken oviduct (54 O.D. units at 260 nm in 2 ml of 10 m*M* Tris–HCl buffer, pH 7.5 containing 0.5 *M* KCl and 0.2% SDS) was applied to 0.2-ml columns of oligo(dT)-gels equilibrated with the same buffer. The adsorbed material was eluted with 2 ml of 1 m*M* Tris–HCl buffer, pH 7.5. The desorbed material which contains poly(A)$^+$-mRNA was immediately precipitated at -20°C by addition of two volumes of ethanol and 16 μl of 5 *M* ammonium acetate. All operations were done in sterile conditions.

	Oligo(dT)-Ultrogel		*Oligo(dT)-Trisacryl*		*Oligo(dT)-cellulose*	
	O.D. units 260 nm	*%*	*O.D. units 260 nm*	*%*	*O.D. units 260 nm*	*%*
Total amount of RNA used	54	100	54	100	54	100
Adsorbed and eluted fractions	0.35	0.66	0.41	0.76	0.49	0.91

The adsorption efficiency expressed as the amount of poly(A) adsorbed per mg of immobilized oligo(dT) is higher for oligo(dT)–Ultrogel and oligo(dT)–Trisacryl than for oligo(dT)–cellulose (Table I). This can be related to a better accessibility to the oligo(dT) immobilized on the very porous matrices (Ultrogel and Trisacryl) than on the cellulose which comprises tightly packed fibres.

Purification of poly(A)$^+$–mRNA

A practical application of the oligo(dT) supports is the purification of poly-(A)$^+$–mRNA from total cellular mRNA. Table II presents an example of the purification of oviduct poly(A)$^+$–mRNA from total RNA extract by affinity chromatography. From 54 O.D.$_{260\ nm}$ units of RNA used, the quantity of RNA adsorbed and eluted from the gels was between 0.35 and 0.49 O.D. units, which represents about 1% of the initial quantity. The main part of the crude RNA was not in fact able to interact with the oligo(dT) chains anchored to the gels and was recovered in the non-adsorbed fraction.

Fig. 2 shows the electrophoresis of the radiolabelled translation proteins obtained using an identical amount of purified poly(A)$^+$–mRNA obtained respectively from oligo(dT)–Ultrogel (C), -Trisacryl (D) and -cellulose (E).

The ability of these mRNA to synthesize ovalbumin is clear when one compares their spot intensities to those obtained with crude oviduct RNA (lane B). The purity of ovalbumin mRNA, being proportional to the intensity of the electrophoretic spots, seems to be higher after chromatography on oligo(dT)-substituted Ultrogel and Trisacryl than on cellulose.

The adsorption capacity, adsorption efficiency, the absence of non-specific adsorption for poly(A) fragments and the purity of mRNA suggest that the new chromatographic sorbents will prove suitable for the isolation of other poly(A)$^+$–mRNA.

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