Fractionation and characterization of polyadenylated RNA from broad bean meristematic root cells

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Abstract: Several populations of polyadenylated RNA from *Vicia faba* meristematic root cells were fractionated by stepwise thermal elution from poly(U)-Sepharose following sequential phenol extraction. Analysis of these fractions showed that the size of the poly(A) segment could influence this fractionation, but in some cases other characteristics of the molecule are involved. Evidence was obtained that 45-60% of the nucleotides of plant polyadenylated RNA are in base paired regions, as was previously demonstrated for mammalian mRNA.

Introduction

The role of the polyadenylic segment present in many, but not all, mRNA is one of the most interesting problems in molecular biology. Studies with animal cells are now suggesting that the poly(A) segment might determine the life of the molecule [1–3]. But, in higher plants, this research is much less advanced. The presence of the poly(A) segment at the 3' end and its mean overall length alone are known. Fractionation of plant polyadenylated RNAs on the basis of poly(A) length may be thus very useful. We have previously described the purification and the fractionation of polyadenylated RNA from *Vicia faba* meristematic root cells by stepwise thermal elution from poly (U)-Sepharose [4]. Contaminating RNA (mainly rRNA) was removed at 30°C and two populations of polyadenylated RNA collected at 40 and 50°C respectively. The results obtained suggested that these two populations differ mainly in poly(A) size. In this paper we present a further structural analysis of these fractions.
Materials and methods

Materials

Broad beans (Vicia faba var. Aguadulce) were obtained from G. Truffaut, Vineuil. [3 H] adenine was obtained from Sigma (U.S.A.). All other chemicals were of reagent grade and obtained from Merck (Darmstadt). Poly(U)-Sepharose was from Pharmacia.

Methods

Preparation of RNA. Preparation of labelled RNA was carried out from excised apices (5 mm) of broad bean roots incubated for 2 h in 20 μC of 3 H adenine/ml (5 apices per ml). Homogenization of apices, sequential extraction of RNA at pH 7.6 in the presence of MgCl2 and KCl and then at pH 9.0 without salt [5], and measurement of radioactivity were performed as described before [6].

Poly(U)-Sepharose chromatography. Binding to poly(U)-Sepharose was done at 25°C in a saline-SDS buffer (0.1 M NaCl, 0.01 M EDTA, 0.01 M Tris-HCl pH 7.4, 0.2% sodium dodecylsulfate). Elution was with the same buffer without NaCl, the temperature being raised successively to 30, 40 and 50°C.

Polyacrylamide gel electrophoresis of RNA and Poly(A)

3% polyacrylamide gels were prepared according to Loening [7] in the presence of 0.5% agarose [8]. RNA and poly(A) were dissolved in electrophoresis buffer containing 50% sucrose. RNA was incubated for 5 min at 50°C in 20% (v/v) formamide before electrophoresis. Electrophoresis was carried out for 2 h at 5 mA/gel and after the run, the gel was frozen and cut into 1 mm slices. Each slice was incubated 24 h at room temperature in a Scintillation and Solubilization mixture from Lumac, France [Lipoluma 10, Lumasolve 1, H2O 0.2 (v/v)].

Determination of the content in poly(A) by the fluorometric method

The titration of polyadenylated RNA with poly(U) in the presence of ethidium bromide for the quantification of poly(A) was according to the method of Favre [9, 10]. The same amount of RNA, intact of after digestion for 1 h with 10 units of RNAse T1 was prepared in 2.10^-6 M ethidium bromide, 0.05 M NaCl, 0.05 M sodium cacodylate buffer pH 7.0. The titration of each sample was performed by the stepwise addition of 2 ml aliquots of 0.5 x 10^-4 M poly(U), and the fluorescence (exc.540 nm; em.600 nm) determined 2 min after each addition. The reference titration of a 0.5 x 10^-4 M synthetic poly(A) solution was conducted in the same conditions. Fluorescence was measured with a Jobin-Yvon spectrofluorimeter. The cuvette containing 500 μl of solution was inserted in a cuvette holder thermostated at 25°C.