DEOXYCYTIDYLATE ANTIBODIES DISTINGUISH DNA FROM RNA

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ABSTRACT

Antibodies elecited against thyroglobulin conjugate of deoxycytidylate bind to denatured DNA and not to RNA or native DNA. The DNA binding is deoxycytidylate specific.

INTRODUCTION

ANTIBODIES raised against protein conjugates of the modified nucleosides m^6A, m^7G and i^6A have been used for the isolation of nucleic acids or oligonucleotides containing the respective nucleoside becuase of the high specificity of the antibodies to the respective base[1-6]. Similarly, antibodies specific to DNA-RNA hybrids have been used for the isolation of rRNA genes after hybridization with rRNA[7].

All the antibodies elicited so far against normal nucleoside and nucleotide haptens have shown reactivity with both denatured DNA[8-10] and RNA[10-12] but not with native DNA[9,13]. In this paper, it is shown that dpC antibodies have a novel specificity in polynucleotide binding, namely, they bind to denatured DNA but not to RNA or native DNA.

MATERIALS AND METHODS

Nitrocellulose filters (0·45 μ) were from Microdevices, Ambala, India. ^{3}H-poly A$^+$ hnRNA was a gift from Dr. K. Jayaraman, Johns Hopkin University, U.S.A. ^{32}P-DNA (specific activity 8577 cpm/μg) was prepared from ^{32}P-labelled colitis phage[11] by phenol extraction[5]. dpC antibodies were elicited in rabbits using thyroglobulin conjugate of deoxycytidylic acid (Tg-dpC) as the immunogen[16]. The dpC specific antibodies were isolated from the antisera by affinity adsorption to aminohexyl-sepharose-dpC column and subsequent elution with dpC (Unpublished results). Purified dpC antibodies were coupled to cyanogen bromide activated sepharose 4B according to the procedure of March *et al.*[17] and used for the separation of DNA and RNA. About 12 mg of dpC antibodies were found incorporated per 10 ml packed volume of sepharose as estimated from the recovery of uncoupled proteins.

DNA was denatured by heating at 100° C for 10 min and chilling rapidly in ice-salt mixture. The binding of ^{32}P-DNA and ^{3}H-RNA to antibodies was studied by nitrocellulose filter method[9].

RESULTS

Figure 1*a* gives the results of the binding of native and denatured ^{32}P-DNA and ^{3}H-RNA to dpC antibodies. Only denatured DNA shows binding. Fig 1*b* shows the binding of denatured ^{32}P-DNA to the antibodies in the presence of non-radioactive deoxyribomononucleotides. For 50% inhibition of the binding dpT and dpA are needed in 100 and 2300 fold concentration respectively, compared to dpC whereas dpG gives negligible inhibition, showing that the DNA binding observed is fairly dpC specific.

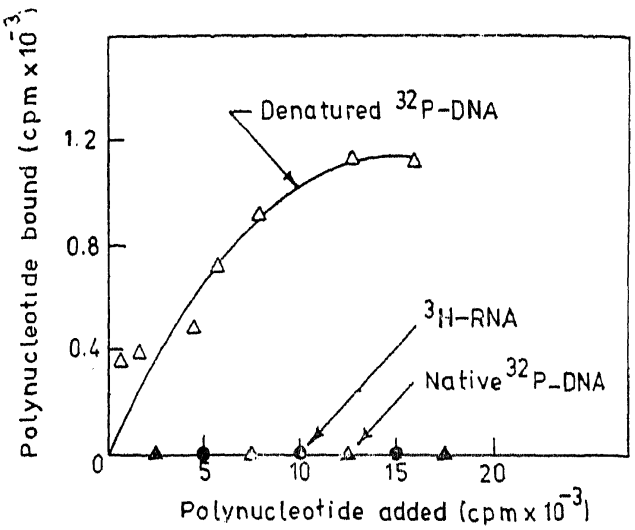

FIG. 1*a*. Binding of labelled polynucleotides to dpC antibodies.

Reaction mixture consisting of purified dpC antibodies (40 μg) and native ^{32}P-DNA or denatured ^{32}P-DNA (varying amounts) in TBS (0·3 ml; 0·01M Tris-HCl, pH 7·5, 0·15 M NaCl and 0·02% NaN_3) was incubated at 37° C for 1 hr and at 4° C for 24 hr and filtered through nitrocellulose filters, the filters washed with 10 ml TBS, dried and counted for radioactivity in 0·5% 2, 5-diphenyloxazole in toluene. Control experiments were done with normal γ-globulins.

^{3}H-RNA binding assays were done as above except that the reaction mixture contained 0·2M Na_2SO_4 also.

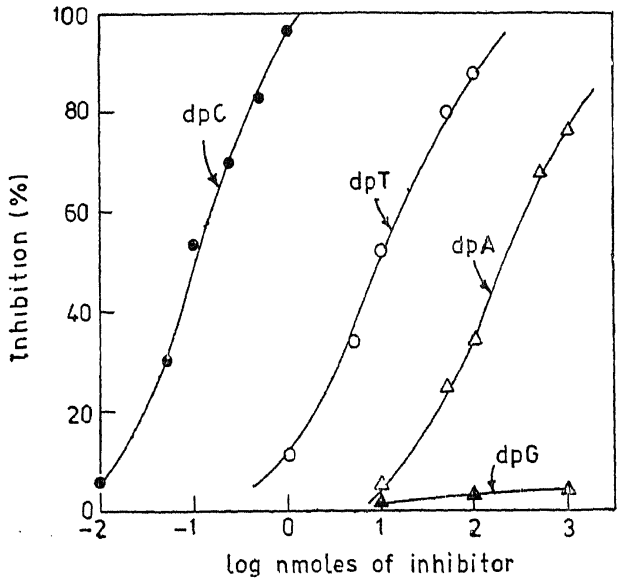

FIG. 1*b*. Inhibition of the binding of denatured ^{32}P-DNA to dpC antibodies.

Reaction mixture consisting of purified dpC antibodies (66·5 μg), heat denatured ^{32}P-DNA (8000 cpm) and inhibitor (varying amounts) in TBS (0·3 ml) was incubated, filtered through nitrocellulose filters and radioactivity counted as in Fig. 1*a*. Control with normal γ-globulins retained 506 cpm of ^{32}P-DNA on the filter. Antibodies without inhibitor bound net 990 cpm of ^{32}P-DNA.

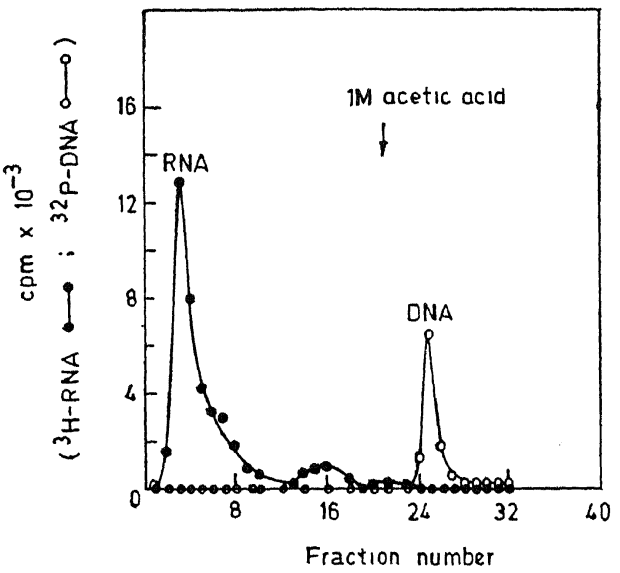

FIG. 2. Immunoadsorption chromatography.

A mixture of denatured ^{32}P-DNA (16, 100 cpm) and ^{3}H-RNA (40, 200 cpm) in TBS (1 ml) was loaded on anti-dpC-sepharose column (6 ml packed volume, 1 cm diameter, pre-equilibrated with TBS), washed with TBS and eluted with 1M acetic acid. Fractions (1·0 ml) were collected from loading onwards. The acetic acid eluted fractions were neutralized with NaOH. 200 μl from each fraction was transferred onto Whatman No. 3 paper circle (25 mm diameter) dried and subjected to differential counting with 0·5% 2,5-diphenyloxazole in toluene (10 ml).

Figure 2 shows the results of the chromatography of denatured ^{32}P-DNA and ^{3}H-RNA on immobilised dpC antibodies. The unbound fraction and the TBS wash contain only ^{3}H-counts and no ^{32}P-counts. Acetic acid eluted fractions contain only ^{32}P-counts. The recovery of ^{32}P-DNA was not quantitative but the recovery of ^{3}H-RNA was quantitative.

Discussion

In the present studies, nitrocellulose filter assay has been used for studying the binding of native DNA, denaturated DNA and RNA to antibodies. This method is normally used for studying the binding of native DNA[18] and RNA[19] to proteins as native DNA and RNA are not retained on nitrocellulose filters but proteins are. Denatured DNA is known to be retained on nitrocellulose filters and the retention is known to vary with the source, the treatments given and the assay conditions[20,21]. We have found that in the case of nitrocellulose filters manufactured by Microdevices (India), the retention of denatured DNA is low enough to be used without any pre-treatment for studying the interaction of antibodies with denatured DNA.

The ability of dpC antibodies to bind to denatured DNA and its inability to bind to RNA and native DNA are established in the present studies by using radioactive DNA and RNA for binding. This property of the antibodies is further demonstrated by using them for the separation of RNA and DNA by immunoadsorption chromatography. It is expected that this novel property of dpC antibodies will prove useful, for example, as a base specific probe for ssDNA regions in chromatin in the presence of RNA.

Abbreviations

m^{6}A, N^{6}-methyladenosine; m^{7}G, 7-methylguanosine; i^{6}A, N^{6}-(Δ^{2}-isopentenyl) adenosine; dpC, deoxycytidine-5′-phosphate; dpT, deoxythymidine-5′-phosphate; dpG, deoxyguanosine-5′-phosphate; dpA, deoxyadenosine-5′-phosphate; ssDNA single stranded DNA; Tg, thyroglobulin.

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TISSUE CULTURE OF THE JACK TREE

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Abstract

Shoot tips isolated from the juvenile shoots of jack (*Artocarpus heterophyllus*) cultured on MS basal medium containing an auxin and a cytokinin resulted in multiple shoot induction through stimulation of axillary buds. The shootlets grew only as shoots in culture. Rooting of isolated shootlets occurred rarely. This method, if copious rooting could be induced on a large scale, points to the possibility of clonal propagation supplementing the conventional methods.

Introduction

In the study of morphogenesis *in vitro*, most herbaceous forms of plants seem to present no problem to their being grown as de-differentiated tissue and in the manipulation of their callus to re-differentiated shoot buds, roots, or complete plants, which is now a routine[8]. This has implications in the rapid clonal propagation, in larger numbers and in quicker time of many ornamental and other horticultural species. A survey of literature pertaining to the study of organ morphogenesis in aseptic culture of the tree species indicates that apart from members of the Coniferae and a few temperate woody angiosperms such as the aspen, elm, poplar, birches, etc., there have been no serious attempts at clonal propagation of the elite tropical fruit trees through tissue culture. This prompted us to investigate the culture conditions that influenced the growth of explanted organs of the jack (*Artocarpus heterophyllus* Lam.), a tree species belonging to the family Moraceae with particular emphasis on multiple shoot induction *in vitro* and rooting of the isolated shootlets to form whole plants. This report is based on such an attempt.

Materials and Methods

Artocarpus sp. is a fairly large-sized tree originally native to India, but now cultivated in Tropical Asia and Africa for its large edible fruits. Propagation is by seed, cuttings or patch buddings, Superior races of the jack tree are known and budlings in general, are difficult to transplant from the nursery row. One of the trees growing at the Indian Institute of Horticultural Research, Bangalore (India) was utilized as a source material for shoot buds and young inflorescence axes for experimentation *in vitro*.

Sprouts from germinating seeds as also slices of young inflorescence axes were used. Shoot tips, cotyledons and hypocotyl fragments were aseptically cultured on nutrient media after sterilization with mercuric chloride (0·2%) for 15 min followed by several rinses in autoclaved distilled water. Murashige and Skoog's[3] basal medium (MS) supplemented with

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