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Short Communication

Purification of common bean chloroplast DNA by DEAE cellulose column chromatography

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ABSTRACT

DEAE cellulose column chromatography has been used for the purification of chloroplast DNA. This technique is more time-efficient than the caesium chloride gradient method. It is a reliable tool and yields a high concentration of double-stranded DNA molecules. The chloroplast DNA obtained after DEAE cellulose chromatography is sensitive to different restriction endonucleases and can be used for DNA fragment cloning and sequencing.

INTRODUCTION

Chloroplast DNA (cpDNA) has been purified on caesium chloride gradients from a variety of plant species after RNase T1 treatment [1]. In general the caesium chloride gradient technique is excellent for the preparation of small amounts of DNA. However, this method is expensive, time-consuming and yields DNA containing considerable amounts of caesium chloride.

RNase T1 treatment is an important step in several methods used in DNA preparation [1,2]. This treatment destroys RNA contaminants but often presents a risk of DNase 1 contamination, which can digest the DNA molecules.

The diethylaminoethyl (DEAE) anion exchanger has been used in the nucleogen–DEAE form as a column support for high-performance liquid chromatography isolation of plasmid DNA [3]. DNA cellulose has also been used for isolating lambda phage [4,5], proteins [6] and tRNA [7,8].

These earlier observations have been used to develop a procedure for purifying chloroplast DNA by DEAE cellulose column chromatography. The chromatographic conditions used here make it possible to separate DNA from RNA in a given chloroplast nucleic acid preparation. The DNA was analysed by endonuclease digestion and agarose gel electrophoresis.

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EXPERIMENTAL

Materials

Bean culture was performed either outside or in a greenhouse. Young bean leaves of *Phaseolus vulgaris*, variety Saxa, were harvested after 15 days for chloroplast preparation and nucleic acid extraction.

Chemicals and reagents

DEAE cellulose, DNase 1, RNase T1, proteinase K and the restriction endonucleases BamHI and XhoI were obtaiend from Sigma (St. Louis, MO, USA). All the reagents used were of analytical-reagent grade and all solutions were made with sterile distilled water.

Chromatographic conditions

The DEAE cellulose was pre-cycled before use according to the instructions of the manufacturer [9]. Dried DEAE cellulose (10 g) was first suspended in 0.5 *M* hydrochloric acid, for 30 min, then subjected to several washes with water, to adjust the solution to pH 7.0 and to remove the fines by decanting. The DEAE cellulose was treated with 0.5 *M* sodium hydroxide and again washed to pH 7.0. The DEAE cellulose was stored in buffer A (0.2 *M* sodium chloride, 0.05 *M* Tris–HCl, pH 7.5) at 4°C.

About 5 ml of the DEAE cellulose were packed into a 20-ml syringe plugged with sterile glass wool and equilibrated with buffer A.

Preparation of chloroplasts

Chloroplasts and chloroplast nucleic acids were prepared by a previously described procedure [10] with some modifications. The following steps were performed at 4°C. Briefly, 1 kg of bean leaves was homogenised in a Waring blender with 2000 ml of a solution containing 5 m*M* Tris–HCl (pH 8.0), 25 m*M* ethylenediaminetetraacetic acid (EDTA), 1.25 *M* sodium chloride, 0.01% bovine serum albumin (BSA) and 7 m*M* 2-mercaptoethanol. The extract was first filtered through one layer of cheesecloth followed by a 50-μm nylon mesh and finally through a 25-μm nylon mesh (Lockertex, Warrington, UK). After centrifuging for 1 min at 3000 *g*, the chloroplasts were recovered in pellet form and stored at −20°C or used immediately for DNA extraction.

Preparation of chloroplast nucleic acids

About 10 g of chloroplasts were suspended in 100 ml of a solution of 10 m*M* Tris–HCl (pH 8.0), 10 m*M* sodium chloride, 1% sodium dodecyl sulphate and 5 mg of proteinase K. The mixture was incubated at 37°C for 12 h.

The suspension was extracted with an equal volume of phenol saturated with $T_{10}E_{10}$ buffer [10 m*M* Tris–HCl (pH 8.0), 10 m*M* EDTA] and the nucleic acids were recovered in the aqueous phase after centrifuging for 20 min at 12 000 *g*. A one tenth volume of 3 *M* sodium acetate (pH 5.4) was added to the aqueous phase and the nucleic acids were precipitated with two volumes of cold 95% ethanol.

The precipitated nucleic acids were recovered in pellet form after centrifuging for 10 min at 10 000 *g*, resuspended in 2 ml of $T_{10}E_1$ buffer [10 m*M* Tris–HCl (pH 7.5), 1 m*M* EDTA] and dialysed.

After dialysis against water for 24 h at 4°C, a one tenth volume of 3 *M* sodium acetate (pH 5.4) was added to the nucleic acid solution and the nucleic acids were reprecipitated with two volumes of ethanol. After centrifugation, the pellet was resuspended in 2 ml of buffer A.

Chromatography of chloroplast nucleic acids

The nucleic acid solution, containing 50 mg of nucleic acids, was loaded onto the DEAE cellulose column and the column was washed with buffer A to remove unbound material which was collected in 2-ml fractions. The UV absorption at 260 nm (A_{260}) of each fraction was measured; peak I was observed, and elution continued until all A_{260} absorbing material had been removed from the column, which required 24 ml of buffer A.

The column-bound nucleic acids were eluted with buffer B [1.0 *M* sodium chloride, 0.05 *M* Tris–HCl (pH 7.5)]. A second A_{260} peak (peak II) was obtained and elution continued until all A_{260} absorbing material had been removed from the column.

The fractions under peaks I and II were pooled, giving volumes of 8 and 14 ml, respectively. Each set of pooled fractions was precipitated with two volumes of ethanol. Each precipitate was washed twice with 70% ethanol to remove NaCl, and the air-dried pellet was dissolved in 0.5 ml of $T_{10}E_1$ buffer (pH 7.5).

The nucleic acids recovered from the DEAE cellulose column were initially characterised by recording the absorption spectrum in the range 220–300 nm on a Shimadzu UV-VIS recording spectrophotometer (Model UV-160A).

Nucleic acid analysis

Both nucleic acids were treated with either DNase 1, RNase T1 or proteinase K. To three Eppendorf tubes the following reagents were added: 10 μl of one of the nucleic acid samples, 2 μl of a 10 × digestion buffer (100 m*M* Tris–HCl pH 7.5, 100 m*M* magnesium chloride, 100 μg/ml BSA, 1000 m*M* sodium chloride) and 6 μl of sterile distilled water. Into tube 1 were added 2 μl of DNase 1 (0.1 μg/μl), tube 2 2 μl of RNase T1 (1U/μl) and tube 3 2 μl of proteinase K (0.05 μg/μl). Tube 1 was incubated at 0°C while tubes 2 and 3 were incubated at 37°C. The tubes were all incubated for 2 h.

DNA was digested with restriction endonucleases. The reaction medium contained 10 μl of cpDNA (0.5 μg/μl), 5 μl of the 10 × digestion buffer, 1 U of either BamHI or XhoI enzyme per μg of cpDNA and sterile distilled water to bring the volume to 50 μl. After incubating the mixture for 3 h at 37°C the DNA fragments were separated by gel electrophoresis in 1% agarose. The bands were visualised after ethidium bromide staining.

RESULTS AND DISCUSSION

The chloroplast extract purified on a DEAE cellulose column contained two different types of nucleic acids, as shown by their chromatographic migration (Fig. 1).

The identification of chloroplast nucleic acid components was obtained after analysis of the nucleic acids peaks and their fractionation on agarose gel electrophoresis. Upon electrophoresis, the large DNA molecules moved more slowly than the

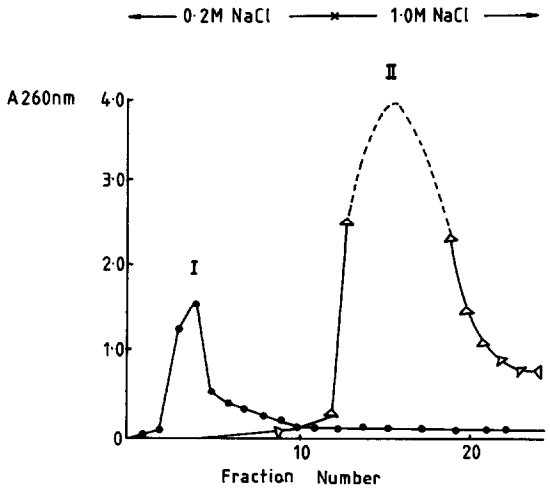

Fig. 1. Elution profile of chloroplast nucleic acids on DEAE cellulose column chromatography. Each fraction has a volume of 2 ml. Peak I is DNA and peak II RNA.

smaller RNA molecules. This indicated that peak I contained only intact DNA while peak II contained RNA and degraded DNA. The RNA, which are tRNA molecules, may be easily purified by polyacrylamide gel electrophoresis with the removal of the degraded DNA fragments [7,8].

The DNA was not tightly bound to DEAE cellulose and was eluted with the 0.2 *M* sodium chloride buffer (Fig. 1, peak I). The more tightly bound RNA was eluted with the 1.0 *M* sodium chloride buffer (Fig. 1, peak II). The DNA and RNA peaks were confirmed by degradation with DNase 1 and RNase T1, respectively. They were not affected by proteinase K treatment.

The sum of the molecular weights of the DNA fragments after XhoI digestion was estimated to be 150 kbp. This value is identical to that reported for common bean chloroplast DNA on a caesium chloride gradient [8,11].

In this work a yield of 50 mg of chloroplast nucleic acids was obtained from 1 kg of common bean leaves. The chloroplast nucleic acids were separated into their two major types, DNA (5 mg) and RNA (30 mg), by DEAE cellulose column chromatography. In deriving these estimates of yield, the relationship whereby one absorbance unit at 260 nm is equivalent to 50 μg/ml DNA and 40 μg/ml RNA was used [2,6]. The A_{260}/A_{280} ratio was about 1.80. This indicates that the recovered DNA and RNA are reasonably pure.

This separation technique has the advantages of being fast, cheap and easy to perform. The DNA recovery is five times higher than that reported for the same weight of higher plant leaves [10].

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