A NEW METHOD OF DETERMINATION OF ADENINE UNITS LOCATION IN DNA

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1. Introduction

We proposed [1,2] a general approach to the determination of sequences of oligo- and polynucleotides based on incomplete chemical splitting of oligo- and polynucleotides labelled on terminal residues followed by the determination of the lengths of all possible labelled products. Later on, after substantial improvement, a similar approach was used by Maxam and Gilbert [3]; at present, together with the Sanger method [4], the Maxam-Gilbert approach is the basic means for determining DNA sequences. Using the Maxam-Gilbert procedure in our work, we found a convenient method for localizing adenine residues based on our method for the incomplete de-adeninisation of the DNA with formaldehyde in the presence of primary amines [5,6]. This technique is described here.

2. Experimental

The *Eco* RI-G DNA fragment from λimm^{434} was obtained in accordance with the technique described [7]. Its sequence has been reported [8,9].

Pharmaceutical formalin (Merck, FRG) served as the source of formaldehyde. Formaldehyde concentration was measured iodometrically. Ethanolamine (Reachim) was purified by distillation before use.

In order to find the location of adenine units in DNA, a fragment labelled with ^{32}P on one of its ends was precipitated with ethyl alcohol; the precipitate, dried in vacuum, was treated with 50 μl of a solution containing 0.4 M formaldehyde and 1.2 M ethanolamine (pH 6.5).

The mixture was incubated at 37°C for different time depending on the length of the sequence under determination. After the incubation the solution was treated with 5 μl 3 M sodium acetate (pH 5.5) and 150 μl ethyl alcohol, cooled at − 70°C for 15 min and centrifuged for 15 min at 10 000 rev./min. The pellet was dissolved in 100 μl 0.3 M sodium acetate and the alcohol precipitation repeated. The newly-formed precipitate was washed with alcohol and dried in vacuum. The partially de-adeninised DNA was then split with piperidine.

3. Results and discussion

During the reaction between formaldehyde and DNA in the presence of primary amines the detachment of adenine residues from the polymeric chain is observed [5,6]. We attempted to utilise this reaction to determine the location of adenine units in the polynucleotide chain by using the method of incomplete de-adeninisation. We tested its applicability on the right part of the *Eco* RI fragment obtained by splitting λimm^{434} phage DNA with *Eco* RI restriction endonuclease. The whole fragment was labelled with [^{32}P]ATP and polynucleotide kinase on its termini, digested with restrictase, and the required fragment isolated by electrophoresis in polyacrylamide gel. The terminal sequence of this fragment is shown in fig.1.

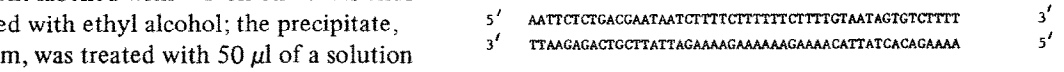

Fig.1. Terminal sequence of a fragment used in checking the de-adeninisation method.

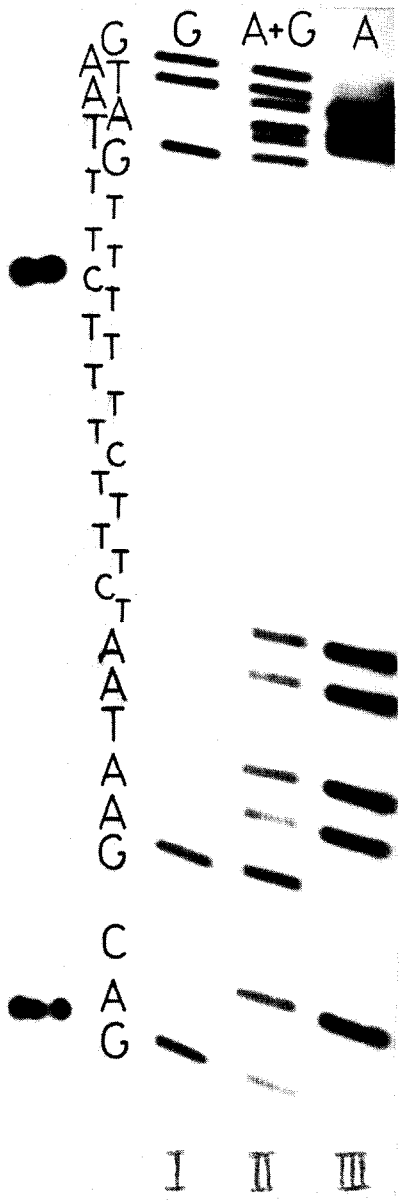

Fig.2. Specificity of DNA splitting at adenine units in ethanol/formaldehyde reaction: (I). Dimethyl sulphate splitting at G units; (II). Formic acid splitting at purine units; (III). Splitting at adenine units by ethanolamine/formaldehyde mixture followed by piperidine treatment.

To evaluate the applicability of the formaldehyde de-adeninisation method for the localization of adenine residues, we compared the results of 3 experiments:

(1) Incomplete specific splitting of the ^{32}P-labelled fragment at guanine units with the use of dimethyl sulphate [3];
(2) Incomplete specific splitting at purine residues with the use of formic acid depurinisation [1,2,8,9];
(3) De-adeninisation by the present method.

It can be seen that in the last case the bands are in an exact agreement with the location of adenine units if piperidine splitting is used after de-adeninisation (fig.2). Thus, the method can be successfully used for determining the location of adenine units.

In conclusion it is necessary to notice two additional points.

1. Our results prove rigorously the high specificity of formaldehyde de-adeninisation in the presence of primary amines, although the mechanism of the reaction has not yet been elucidated.
2. The need for piperidine in splitting de-adeninised DNA might mean that de-adeninisation gives rise not to free deoxyribosyl residues but to a deoxyribose derivative, probably, a product of reaction with a primary amine.

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