A RESTRICTION ENDONUCLEASE FROM *AGROBACTERIUM TUMEFACIENS*

Gérard ROIZÈS

Equipe de Recherche de Biophysique du CNRS, Université des Sciences et Techniques du Languedoc, 34060 Montpellier

and

Marcel PATILLON and Aries KOVOOR

Laboratoire de Physiologie de la Différenciation Cellulaire Végétale, Université Paris VII, 75005 Paris, France

Received 27 June 1977

1. Introduction

The tumorous growth of plant tissues known as Crown-gall is induced by certain strains of *Agrobacterium tumefaciens* (Smith and Townsend, Conn.) and there is considerable evidence that foreign DNA is integrated in the genome of the transformed host cell [1]. A specific endonuclease of the bacterium could thus conceivably play a rôle in the insertion process.

In this letter we describe the isolation of a sequence-specific endonuclease from a tumorigenic strain, B_6, of *Agrobacterium tumefaciens*. The recognition site as well as the modification specificity of this enzyme, named *Atu*I, are shown to be identical to those of a known restriction enzyme, *Eco*RII.

2. Methods and results

2.1. *Preparation of* Atu*I*

Bacterial cells (20 g) in late exponential phase were disrupted by sonication in 30 ml 0.01 M Tris–HCl (pH 7.5) containing 0.01 M β-mercaptoethanol and centrifuged at 100 000 × *g* for 90 min. The supernatant, brought to 1 M NaCl, was fractionated on a Bio-Gel A 0.5 m (Biorad) column (2 × 60 cm) in the same buffer containing 1 M NaCl. All fractions were assayed for nuclease activity on phage λ DNA and the digestion products analysed by agarose slab-gel electrophoresis. Fractions showing endonucleolytic activity in the second of the three 280 nm absorbance peaks were pooled, dialysed against the PC buffer of Roberts et al. [2], applied to a DEAE-cellulose (Whatman DE 52) column (1.2 × 25 cm) and eluted over 200 ml of a linear 0–0.5 M KCl gradient in the same buffer. The combined active fractions eluting between 0.15 M and 0.25 M KCl were redialysed against PC buffer and further submitted to phospho-cellulose (Whatman P 11) chromatography on a column (0.9 × 12 cm) eluted with a 0–0.6 M KCl gradient of 150 ml. The enzyme, with peak activity eluting around 0.4 M KCl, was dialysed against PC buffer containing 50% glycerol. The banding patterns after long incubation times suggest that the enzyme is free of exonuclease activity.

2.2. *Recognition site of* Atu*I*

Digestion of λ, SV40, and vaccinia virus DNAs by *Atu*I yields characteristic agarose gel bands which indicate the enzyme to be a sequence-specific endonuclease. When compared to the bands given by a number of other known restriction endonucleases the patterns were identical to those produced by *Eco*RII (fig.1). We therefore conclude that the recognition site of *Atu*I is the same as that of *Eco*RII, namely,

5′ . . . C–C–T–G–G . . . 3′
3′ . . . G–G–A–C–C . . . 5′

as established by others [3,4].

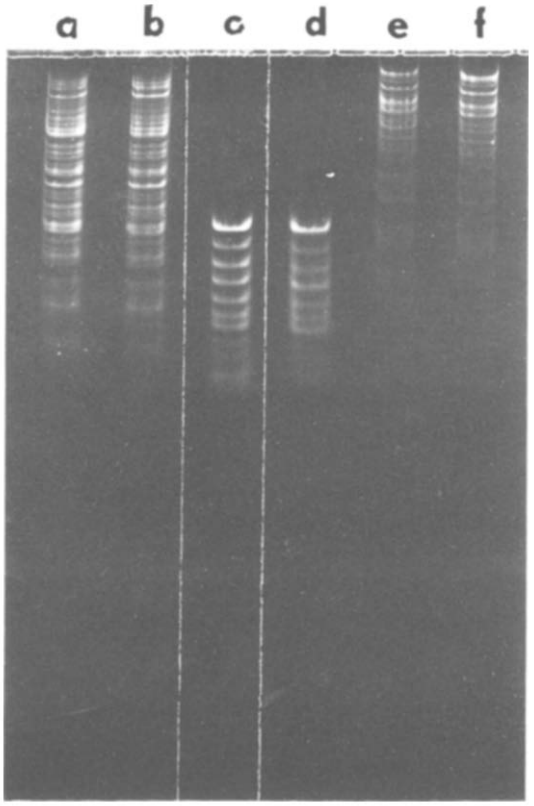

Fig. 1. Digestion products of λ, SV40 and vaccinia virus DNAs by *Atu*I and *Eco*RII. λ, SV40 and vaccinia virus DNAs were incubated overnight with enzyme in a medium containing 6 mM each of Tris (pH 8.0), $MgCl_2$ and β-mercaptoethanol. Digestion products were electrophoresed in a 1.4% agarose gel slab with ethidium bromide (0.5 μg/ml) and photographed (Ilford FP4 film) under ultraviolet light. Lanes(a) and(b), (c) and (d) and (e) and (f) carry respectively λ, SV40 and vaccinia virus DNA hydrolysed separately by *Atu*I and *Eco*RII.

2.3. *Comparison of the modification specificity with that of* Eco*RII*

As expected, when DNA extracted from *Agrobacterium tumefaciens* was treated with *Atu*I no degradation was discernible from the agarose gel pattern, showing the DNA to be appropriately modified. The same DNA was also able to resist attack by *Eco*RII. Consequently, both *Atu*I and *Eco*RII could possibly have the same modification specificity as well.

3. Discussion

The fact that *Atu*I and *Eco*RII are isoschizomers and resemble each other in chromatographic behaviour during the various steps of isolation [5] indicates that the structures of both enzymes are closely related, if not identical. This is noteworthy in view of the taxonomic distance between the two bacterial species. It remains to be seen, however, whether determination of the 5′ ends of the products would show a possible difference in the precise cleavage sites of the respective enzymes.

Elucidation of an eventual involvement of *Atu*I in the induction of plant tumours requires, however, answers to further questions. Thus, as in the case of *Eco*RII, which is associated with a restriction-modification system known to be carried by a drug resistance transfer plasmid [6], *Atu*I could also be plasmid dependent. Indeed, virulent strains of *Agrobacterium tumefaciens*, like the one used in the present study, do contain plasmids, one of which strongly correlates with tumorigenicity [7,8]. An immediate point to be cleared is, therefore, whether *Atu*I is present in non-virulent or cured strains of this bacterium.

Acknowledgements

We wish to thank Dr G. Jaurreguibery for a gift of vaccinia virus DNA and Mr Jacques Martin for excellent technical assistance.

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