AQU I: A MORE EASILY PURIFIED ISOSCHIZOMER OF *AVA* I

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1. Introduction

Since its first description by Murray and coworkers [1] the restriction endonuclease *Ava* I, which recognizes and cleaves at the sequence C↓PyCGPuG, has found wide use in DNA sequence studies. There are, however, three difficulties associated with preparation of this enzyme from its usual source, *Anabaena variabilis* (Kützing). This sheathed filamentous cyanobacterium does not readily yield single colonies on agar plates and is thus not easily rid of contaminating bacteria. It grows slowly (generation times of the order of 24 h). Furthermore, it contains two additional restriction endonucleases, *Ava* II and *Ava* III [1,2]. We frequently find commercial preparations of *Ava* I to be contaminated with one of these additional enzymes, and Roizes et al. [2] were unable to separate *Ava* I and *Ava* III.

We report here that *Agmenellum quadruplicatum* (strain PR-6) produces an isoschizomer of *Ava* I (*Aqu* I). This unicellular cyanobacterium is readily maintained in axenic condition, grows rapidly (4–5 h generation time at 37°C) and contains only this single restriction endonuclease activity.

2. Materials and methods

Agmenellum quadruplicatum (strain PR-6) was grown as in [3]. Cells collected by centrifugation from late stationary phase cultures were washed and resuspended in extraction buffer (EB; 10 mM potassium phosphate (pH 7.0), 1 mM disodium EDTA [(ethylenedinitrilo) tetraacetate], 7 mM 2-mercaptoethanol) supplemented with 0.4 M NaCl and 25 μg/ml phenylmethyl sulfonylfluoride (3 ml buffer for each g wet wt). After 4 cycles of freezing and thawing, cell suspensions were passed twice through an Aminco French pressure cell at 16 000 lb. in^{-2} and subjected to four 30 s sonications at 0°C with a Branson Sonifier. After centrifugation at 39 000 × *g* for 1 h, supernatant fluids were diluted with 1 vol. EB and loaded onto columns (bed vol. ~200 ml or equivalent to that of diluted crude lysate) of phosphocellulose (Bethesda Res. Labs.) equilibrated with EB containing 0.2 M NaCl. After washing with 4 bed vol. of this buffer, bound proteins were eluted with 0.2–1.0 M linear NaCl gradients in EB (3–4 bed vol.). Fractions showing activity were pooled and dialyzed for 48 h at 4°C against 2 l EB (3–4 changes of buffer) and loaded directly onto DEAE-cellulose (Bethesda Res. Labs.) columns (50–200 ml) equilibrated with EB. Columns were washed with 2–3 bed vol. EB and proteins were eluted with linear 0–1.0 M NaCl gradients (in EB, 3–4 bed vol.). Pooled and dialyzed active fractions were loaded directly onto 100–200 ml columns of BioRex 70 (BioRad) equilibrated with EB. After washing with 2–3 bed vol. EB, proteins were eluted with linear 0–1.0 M NaCl gradients (in EB, 3–4 bed vol.). Peak fractions were pooled, dialyzed, concentrated by burying dialysis bags in dry Sephadex G-100 (Pharmacia), and stored at 4°C.

For restriction endonuclease assays, 10 μl portions of column fractions were added to 40 μl λ buffer mix (1 μg λ DNA (Bethesda Res. Labs.) in 100 mM Tris (tris (hydroxymethyl)aminomethane)–HCl (pH 7.5), 5 mM $MgCl_2$, 100 mg/ml bovine serum albumin, 7 mM 2-mercaptoethanol). After 1–4 h incubation at 37°C, reactions were stopped and samples were loaded into the wells of horizontal 1.0% agarose slab gels [3,4].

3. Results and discussion

Fig.1 shows a phosphocellulose column elution profile of a crude *A. quadruplicatum* lysate, activity

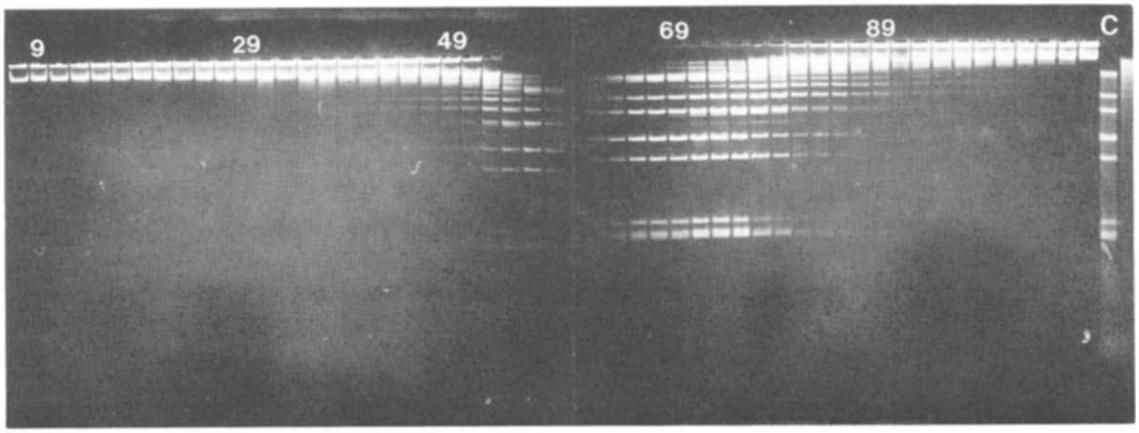

Fig.1. Phosphocellulose column elution profile of crude *A quadruplicatum* lysate assayed against λ DNA. Track C shows digestion profile obtained with 10 μl crude lysate before loading onto column.

being assayed against λ DNA. (There was sufficient activity in 10 μl of the crude extract (corresponding to 3 μg wet cell wt) to give complete cleavage (fig.1C), but it is obvious that such crude lysates also contain non-specific nucleases.) The majority of the activity eluted between fractions 53 and 81. There was no evidence (in this or other such experiments) for a second activity in these fractions or in fractions eluting before or after. The multiple bands observed in fractions 43–57 and 69–99 are readily interpretable as incomplete digestion products. Further purification was effected as in section 2, although it is possible that the phosphocellulose-purified material is of sufficient purity for many purposes.

Fig.2 shows cleavage patterns obtained with λ, φX174, adenovirus 2, SV-40 and pBR322 DNAs (all from Bethesda Res. Labs.), using commercial *Ava* I (New England Biolabs, lot 6), *Aqu* I purified as above, and a third *Ava* I-like activity similarly purified from *A. variabilis* ATCC 29413. (This latter strain, obtained from P. Wolk, appears not to be identical to that used in [1], since it contains only *Ava* I-like activity and a second, minor, activity which appears to be like neither *Ava* II nor *Ava* III [R. H. Lau, unpublished].) Cleavage patterns were identical, although the commercial *Ava* I preparation and the *A. variabilis* ATCC 29413 enzyme contained a contaminating enzyme capable of cleaving SV-40 DNA, which lacks *Ava* I recognition sites [5]. The superior purity of *Aqu* I prepared as described here is more graphically illustrated in fig.3. Approximately equal numbers of units of commercial *Ava* I (New England Biolabs, lot 5) and *Aqu* I were incubated, separately or together, with λ DNA for 0.5, 1, 2, 4, 8 and 24 h. A contaminating activity was apparent after 4 h incubation with the commercial enzyme (or with the mixture), while there was no change in the cleavage pattern produced by *Aqu* I for up to 24 h.

An ~2.6 megadalton fragment resulting from complete digestion of λ DNA with *Ava* I (New England Biolabs, lot 6) and an ~3.3 megadalton fragment resulting from complete digestion of λ DNA with

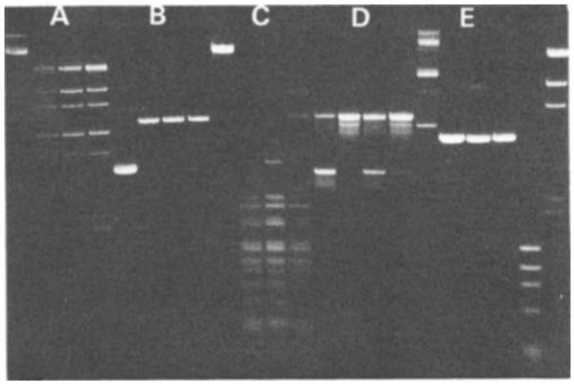

Fig.2. Digestion pattern obtained with λ DNA (A), φX174 RF DNA (B), adenovirus-2 DNA (C), SV-40 DNA (D) and pBR322 DNA (E). For each DNA, the 4 digestion patterns shown are (left–right): that obtained with no enzyme; that obtained with commercial *Ava* I (New England Biolabs lot 6); that obtained with *Aqu* I purified as described; and that obtained with a similarly purified *Ava* I-like enzyme from *A. variabilis* ATCC 29413. The rightmost two lanes are *Hae* III and *Hin*dIII digest of λ DNA.

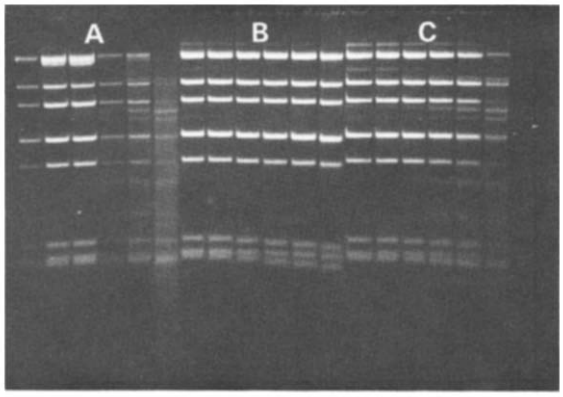

Fig.3. Digestion of λ DNA with approximately equal numbers of units of (A) commercial *Ava* I (New England Biolabs lot 5), (B) *Aqu* I and (C) the two enzymes together. The 6 lanes in each case show (left–right) digestion products obtained after 0.5, 1, 2, 4, 8 and 24 h incubation.

Aqu I were recovered from 1.0% agarose gels by electroelution [3] and ligated separately or together with DNA ligase (Bethesda Res. Labs., ~1 unit in a 20 μl reaction mixture containing ~1 μg DNA, 66 mM Tris–HCl (pH 7.6), 6.6 mM $MgCl_2$, 10 mM dithiothreitol and 0.4 mM ATP, 24 h incubation at 16°C). Ligated *Ava* I-generated 2.6 megadalton fragments (fig.4A) yielded the expected ~5.2 megadalton product. Ligated *Aqu* I-generated 3.3 megadalton fragments (fig.4C) yielded the expected ~6.6 megadalton product. The mixture of both *Ava* I and *Aqu* I fragments (fig.4B) yielded both these products of self-ligation as well as a fragment of ~5.9 megadalton, the product of ligation of the 2.6 and 3.3 fragments. Such a result is expected if *Aqu* I not only recognizes the same sequence as *Ava* I, but also cleaves it in the same position.

Ava I-like activities have been detected in at least 3 strains of *Anabaena variabilis* ([5], and unpublished data cited above for *A. variabilis* ATCC 29413). Each of these strains contains additional restriction endonucleases, grows slowly and is not easily rid of bacterial contamination. *Agmenellum quadruplicatum* (strain PR-6) suffers none of these deficiencies and appears therefore to be a superior source for an enzyme (*Aqu* I) with recognition and cutting specificities identical to those of *Ava* I.

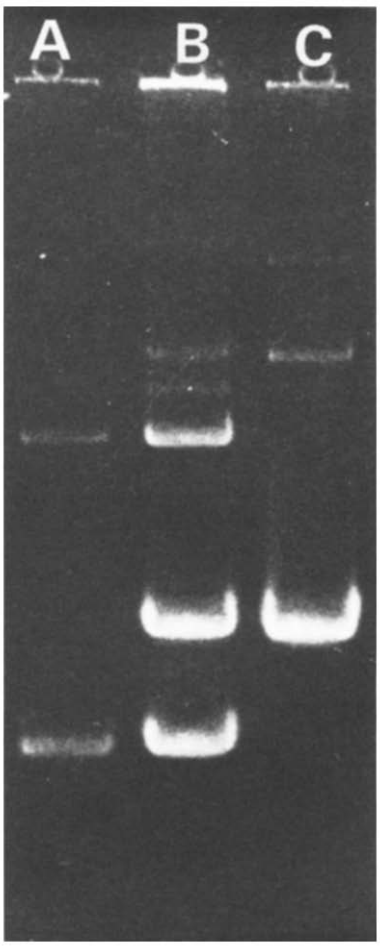

Fig.4. Products of ligation of (A) *Ava* I-generated 2.6 megadalton λ DNA fragments, (C) *Aqu* I-generated 3.3 megadalton λ DNA fragments and (B) a mixture of the two.

Acknowledgements

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References

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