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Short communication

# Use of voltage gradient gel electrophoresis in apoptotic DNA analysis

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**Abstract**

In this paper the use of voltage gradient gel electrophoresis (VGGE) in the electrophoretic analysis of apoptotic DNAs is described. The peculiarity of VGGE fractionation in enhancing DNA bands in the gel by reducing their thickness was used to obtain a rapid, more selective and higher-quality electrophoretic fractionation of apoptotic DNA with respect to conventional electrophoresis. The use of VGGE fractionations also allowed a reduced amount of DNA to be used to detect a characteristic apoptotic DNA ladder pattern, in a lower agarose gel concentration, with respect to conventional electrophoretic fractionation 

*Keywords:* Voltage gradient gel electrophoresis; DNA

## 1. Introduction

Programmed cell death or apoptosis is an endonuclease-mediated process which proceeds through an ordered series of chromatin degradation stages, eventually producing the oligonucleosome fragments that are detected as a characteristic DNA ladder by conventional gel electrophoresis [1].

In this paper we report the use of voltage gradient gel electrophoresis (VGGE) [2,3] for very rapid, high-quality fractionation of apoptotic DNA fragments using gels of relatively low agarose concentration loaded with reduced amounts of analyzed DNA.

We previously demonstrated [2] that VGGE, through a very simple apparatus, creates a decreasing current gradient along the gel (Fig. 1) in electrophoretic fractionation of nucleic acids in an agarose matrix. Moreover VGGE fractionations enhance the sharpness of nucleic acid bands by reducing their thickness [4], and this causes an increase in DNA concentration per gel area unit [4]. This allows detection of lower DNA amounts by UV light as compared to conventional electrophoresis.

Here we examine DNA extracted from both the esophagous and the cutis of the millepede *Enologus oxypygum* (Miriapoda) [5]. Esophagous DNA shows an oligonucleosomal electrophoretic pattern that we

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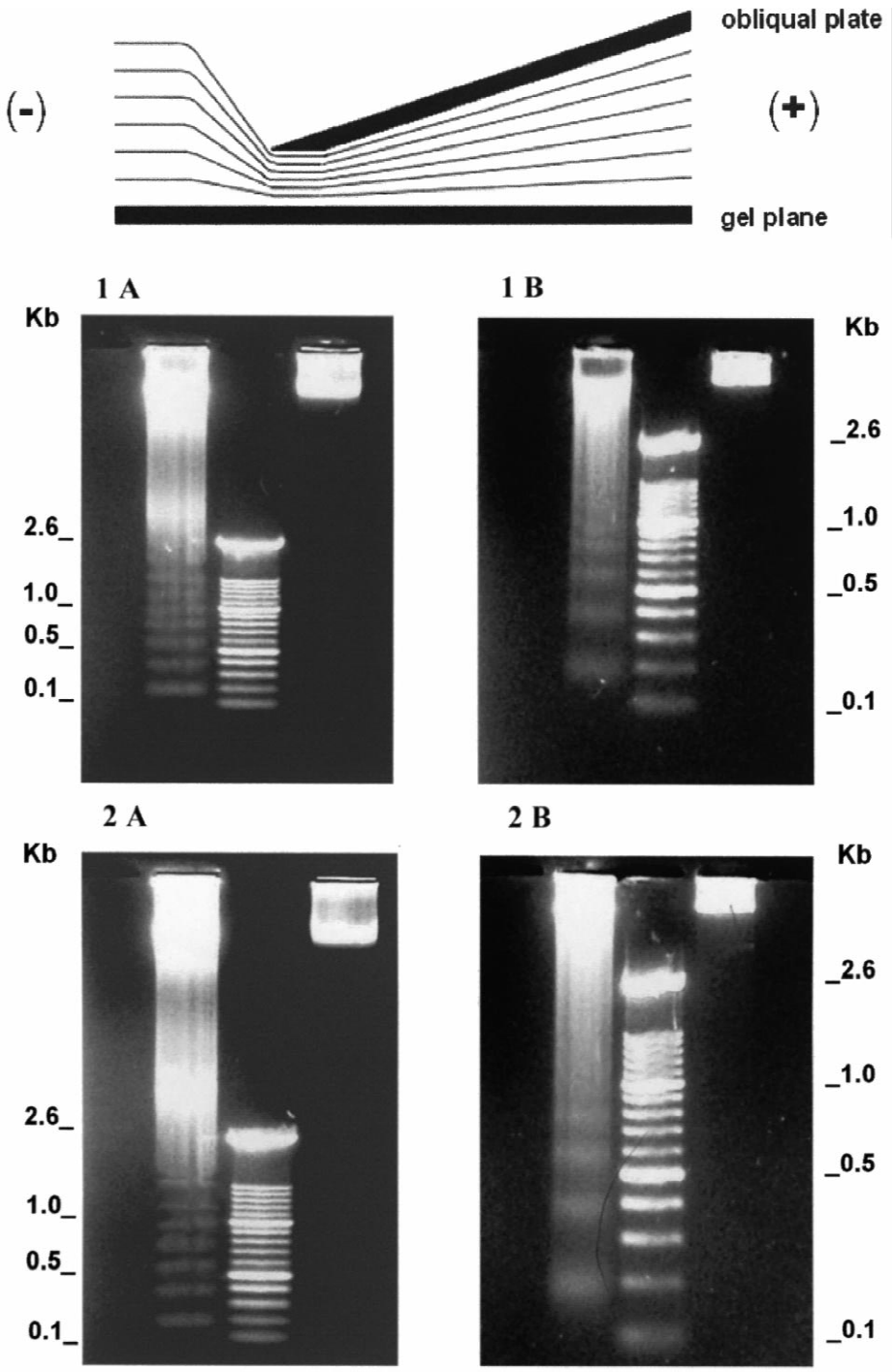


Fig. 1. Schematic drawing of the decreasing current flux in VGGE fractionation. Local electric field progressively decreases as molecules move toward the anode.

Fig. 2. ''Short runs'' (1) and ''long runs'' (2) (for details see the Results and Discussion section) of VGGE (A) and conventional (B) fractionations of DNAs extracted from the esophagous (left tracks) and the cutis (right tracks) of the millepede *E. oxypygum*. A 100-bp ladder DNA marker was also loaded in each gel (central tracks) (Kb=kilobase pair).

interpreted to be a consequence of apoptosis, while DNA extracted from a different tissue (i.e., cutis) is undegraded.

## 2. Experimental

*Enologus oxypygum* DNA was extracted from both cutis and esophagous using a conventional DNA extraction procedure [6]. Tissues were removed with a razor blade, and washed three times in 0.3% NaCl cold solution before dropping them into liquid nitrogen and reducing them to a powder in a Waring blender.

Electrophoretic fractionations were all performed at 3 V/cm in TAE 1× buffer (40 m*M* Tris–acetate, pH 8.0; 2 m*M* EDTA, pH 8.0 [6]) in the same electrophoretic tank. Fractionation of *E. oxypygum* DNA was carried out either in 2.0% agarose gels for conventional electrophoresis or 1.2% gels for VGGE. In all the gels used in our experiments 0.3 mg/ml of ethidium bromide was added. The gels were photographed under UV light.

DNA apoptotic bands were visualized by loading 3.0 μg and 4,0 μg of esophagous DNA in VGGE and in conventional fractionations, respectively. A 3.5-μg amount of cutis DNA was loaded in the gel in both VGGE and conventional fractionations. A 100-base pair (bp) ladder DNA (DNA marker XIV, Boehringer, Mannheim, Germany; 300 ng in all electrophoretic fractionations performed) was used as molecular mass marker.

## 3. Results and discussion

Fig. 2 shows the migration patterns of the same DNAs fractionated using VGGE (A) and conventional electrophoresis (B). To compare VGGE and conventional electrophoretic patterns, a constant migration distance of the 100-bp DNA marker band of 5.4 cm (1A and 1B) in ''short runs'' and of 7.3 cm (2A and 2B) in ''long runs'' were chosen. These last ''long runs'' were performed to demonstrate the persistency of the quality of VGGE fractionation in longer DNA migrations. It is evident from the results that after 75 min and 130 min, VGGE migration patterns (1A and 2A, respectively) are much more selective in apoptotic bands fractionation, when compared to 270 min and 360 min with conventional fractionations (1B and 2B, respectively). Seven, well separated, apoptotic DNA bands are visible in both VGGE fractionation patterns, while the correspondent conventional fractionations reveal no more than five smeared apoptotic DNA bands. Indeed, as the sharpness of the apoptotic bands in VGGE fractionations is much more enhanced with respect to conventional electrophoresis, a reduced amount of DNA was needed to reveal apoptotic phenomena using VGGE (3 μg, with respect to 4.0 μg of the same DNA used in conventional electrophoretic analysis).

Although *E. oxypygum* DNA from the esophagous was not extracted according to a conventional DNA extraction procedure suggested for the analysis of apoptotic DNA [7], we are confident that the DNA ladder we have observed in esophagous DNA fractionations is a consequence of apoptotic phenomena because of both the characteristic DNA fragments lengths we obtained and the reproducibility of the DNA fractionation patterns obtained in many different extractions. Indeed, whatever the origin of this phenomenon, this does not invalidate our results, as its electrophoretic behavior is typical of apoptotic DNAs.

Two strong smears are visible in VGGE fractionated DNAs shown in Fig. 2 (1A and 1B, left tracks), while in the corresponding conventional fractionations only one smeared band is present. As we extracted DNA from *E. oxypygum*-isolated nuclei using a conventional extraction procedure, we interpreted these smears as undigested chromosomal DNA (upper band) and partially digested, medium-molecular-mass DNA fragments (lower band) which were previously described to occur in apoptotic DNA degradation [8].

In conclusion, because of the reasons discussed above, we strongly suggest the use of VGGE fractionation in apoptotic DNA electrophoretic analysis.

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## References

[1] A.H. Wyllie, Nature 284 (1980) 555.
[2] R. Barbieri, V. Izzo, M.A. Costa, G. Giudice, G. Duro, Anal. Biochem. 212 (1993) 168.
[3] M.R. Asaro, V. Izzo, R. Barbieri, J. Chromatogr. A 855 (1999) 723.
[4] R. Barbieri, G. Duro, V. Izzo, Electrophoresis 19 (1998) 643.
[5] R. Vitturi, M.S. Colomba, V. Caputo, I. Sparacio, R. Barbieri, Chromosome Res. 5 (1997) 407.
[6] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning – A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989.
[7] G.M. Yan, S.Z. Lin, R.P. Irvin, S.M. Paul, J. Neurochem. 65 (1995) 2425.
[8] P.R. Walker, J. Leblanc, B. Smith, S. Pandey, M. Sikorska, Methods: Companion Methods Enzymol. 17 (1999) 329.