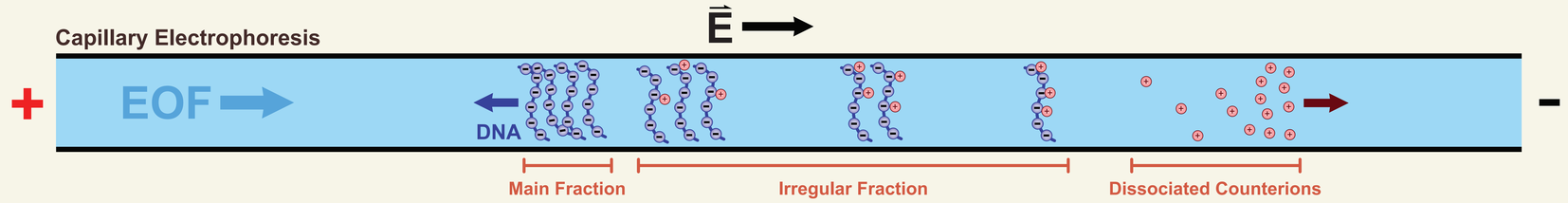
Non-Uniform Electrophoretic Velocity of Homogeneous DNA in a Uniform Electric Field

Michael U. Musheev, Mirzo Kanoatov, Sergey N. Krylov

Department of Chemistry, York University, Toronto, Canada.

Abstract

Electrophoresis-based methods are essential in analysis of DNA. All electrophoretic techniques assume that, within a uniform electric field, identical DNA molecules migrate with a uniform velocity. Here we challenge this assumption, by showing that a small fraction of molecules in a homogeneous DNA sample moves with irregular velocities that vary within a multiple-fold range. We show that this velocity heterogeneity is due to formation and dissociation of stable DNA-counterion complexes.



Introduction

- DNA electrophoretic mobility is defined by its length and conformation
- **Assumption:** In a uniform electric field, within a uniform electrolyte, identical DNA molecules migrate with a uniform velocity (with corrections for diffusion)
- Appears to be true when detected by optical methods like fluorescence **(Fig. 1)**

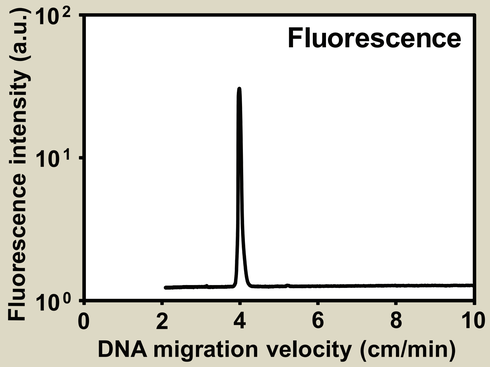


Fig.1. Detection by laser-induced fluorescence reveals a uniform DNA velocity profile.

- **Problem:** High DNA background reported when DNA electrophoresis products are subjected to PCR[1]
- Optical methods have an insufficient LOD to reveal a detailed DNA velocity profile
- Quantitative PCR (qPCR) has a much lower LOD, and a much wider dynamic range.

Goals

1. Reveal detailed electrophoretic velocity profile of DNA
2. Determine the cause of the DNA background
3. Eliminate the DNA background

Methods

- Capillary Electrophoresis with laser-induced fluorescence detector (P/ACE MDQ, Beckman Coulter)
- 10 μM of homogeneous fluorescein-labeled 80-nt ssDNA sample, injected as a short plug
- Sample/Run buffer: 50 mM Tris-Acetate at pH 8.3
- 80-cm, 75 μm inner diameter, bare-silica capillary
- 375 V/cm electric field
- Capillary output collected into one-minute-long fractions
- DNA in each fraction quantitated by qPCR (off-line)

Results

1. Electrophoretic Velocity profile of DNA

- qPCR detection **(Fig. 2)** revealed:
  1. Majority of DNA migrated with a uniform velocity (main fraction)
  2. A small fraction of DNA migrated with a wide range of velocities (irregular fraction)
  3. Irregular fraction was under LOD of laser-induced fluorescence

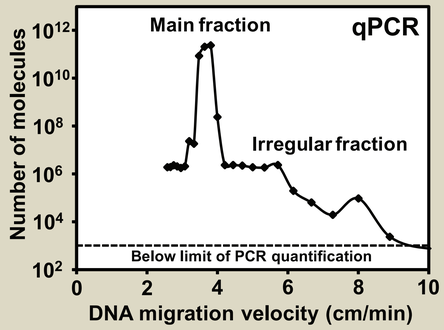


Fig.2. Detection by qPCR reveals a non-uniform DNA velocity profile of ssDNA.

2. Cause of the DNA velocity non-uniformity

☒ **DNA "friction" against capillary walls**
- The irregular fraction was **faster** than the bulk of DNA
- The irregular fraction was **slower** under opposite polarity and suppressed EOF (not shown)

☒ **Anti-stacking**
- Sample Buffer = Run Buffer
- DNA concentration negligible compared to concentration of electrolyte

☒ **DNA conformation**
- dsDNA (double-helix conformation) presented similar velocity profile **(Fig. 3)** to ssDNA (variety of conformations)

☑ **DNA interactions with counterions**
- DNA velocity profile resembled product of dissociation of a stable intermolecular complex
- Majority of DNA counterions form weak "diffusive" complexes
- A small portion of DNA counterions form stable "condensation" complexes[2]

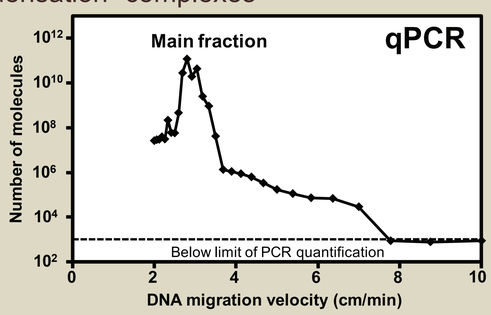


Fig.3. Detection by qPCR reveals a non-uniform DNA velocity profile of dsDNA.

- **Hypothesis:** Non-uniformity of DNA velocity is due to dissociation of condensed counterion-DNA complexes
- **Experiment:** Test effect of counterion concentration on DNA velocity profile
- **Result:** Increasing counterion concentration increases the size of the irregular fraction above fluorescence LOD **(Fig. 4)**

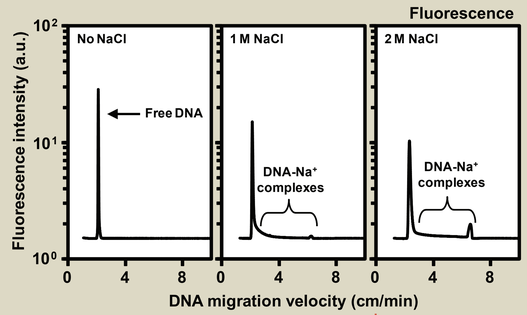


Fig.4. Increasing concentration of Na^+ DNA counterions significantly increases the amount of DNA in the irregular fraction.

- Passive dialysis does not decrease the size of the irregular fraction **(Fig. 5A, blue trace)**, as it cannot remove condensed counterions
- Depletion of counterions by electro-dialysis decreases the size of the irregular fraction **(Fig. 5A, black trace)**
- Re-introduction of counterions into electro-dialysed DNA sample restores the size of the irregular fraction **(Fig. 5B)**

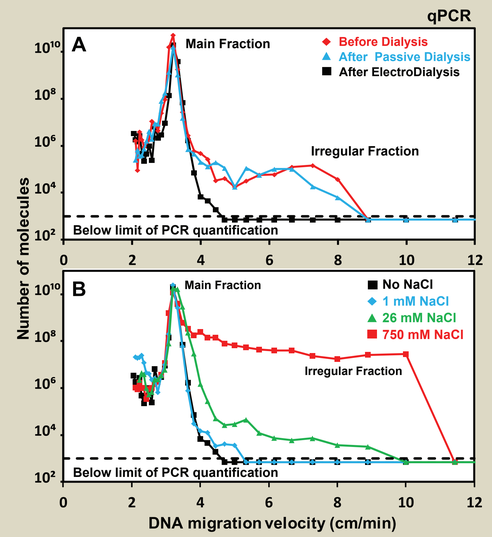


Fig.5. Depletion of counterions decreases the size of the irregular fraction of DNA, while their re-introduction restores it.

Conclusions and References

- Non-uniform DNA velocity profile is caused by electric field-induced dissociation of condensed counterion-DNA complexes
- Associated DNA background in electrophoresis can be reduced by depleting condensed DNA counterions using electro-dialysis.

(1) Berezovski, M.; Drabovich, A.; Krylova, S. M.; Musheev, M.; Okhonin, V.; Petrov, A.; Krylov, S. N. J. .Am. Chem. Soc. 2005, 127, 3165-3171.
(2) Manning, G. S. J. Chem. Phys. 1969, 51, 924-933