Eur Biophys J (2000) 29: 61–65

BIOPHYSICS LETTER

W. Baumgartner · V.P. Pastushenko

Steady-state gel electrophoresis of long polymer molecules: a theoretical study

Received: 10 May 1999 / Revised version: 17 November 1999 / Accepted: 6 December 1999

Abstract The velocity of long polymer molecules in a gel and the liquid flow profile in the vicinity of a molecule's surface were studied theoretically by combining the Navier-Stokes equation with the Poisson-Boltzmann equation. The electrophoretic mobility has been calculated in dependence of the ionic strength of the electrolyte solution, its viscosity, the gels' volume friction coefficient, the surface charge and the radius of the polymer molecule. The results are presented in a non-dimensional form and depend on two dimensionless parameters only. The first parameter is the radius of the polymer molecule in units of the Debye length. The second is a parameter comprising the electrolyte's viscosity and the gel density. Thus, by similarity theory, the results apply to any given experimental arrangement.

Key words DNA · Pulsed field electrophoresis · Mobility · Electrophoretic mobility

Introduction

Gel electrophoresis, especially pulsed field gel electrophoresis (PFGE) (Cantor et al. 1988; Sambrook et al. 1989) is one of the most important tools in modern analytical biochemistry and molecular biology. This role makes necessary a theoretical understanding of the electrophoretic movement of long polymer molecules in gels. Several attempts have been made to describe the electrophoretic movement of polymers, all calculating the electrophoretic velocity by dividing the Coulomb force acting on the charged molecule by a phenomenological coefficient (Lumpkin et al. 1985; Slater et al. 1987; Sambrook et al. 1989) which is typically termed viscous friction coefficient. Unfortunately, the dependence of this phenomenological coefficient on physical properties e.g. the ionic strength of the buffer, its viscosity or the gel density, are not simple to describe. A combined hydrodynamical-electrochemical model of the electrophoretic mobility in gels and the average liquid flow profile near the molecules' surface from the abovementioned parameters has been described (Pastushenko 1991a, b). However, in this study the diameter of the polymer molecule was assumed much larger than the Debye length $1/\kappa$ (i.e. the diffuse ion layer thickness) of the surrounding electrolyte solution. Under physiological buffer conditions the Debye length is of the order of 1 nm, thus it is comparable to the diameter of DNA, single coiled proteins, or uncoiled proteins.

The aim of the present study was to derive an expression for the electrophoretic mobility of a long (in comparison to the mesh size of the gel and the Debye length), cylindrical, charged molecule (e.g. DNA) in a gel of uncharged fibres under the influence of an homogeneous external electric field. For this purpose we considered the steady state movement, assuming the molecule to be oriented along the direction of the homogeneous electric field. This allows us to analyze the indirect influence of gel fibers due to hydrodynamic coupling during gel electrophoresis. The expression for the liquid velocity profile around the molecule has been calculated. This was obtained from the balance of the electrostatic and viscous forces acting in the liquid.

Statement of the problem

To describe the liquid flow near the polymer molecules' surface, one has to combine the electrostatic and the hydrodynamic description of the molecule in the surrounding electrolyte. The electrostatic problem can be described as follows (Pastushenko and Donath 1976a; Pastushenko 1991a). A long molecule of radius a and a surface charge density σ is put into a gel with a

---

W. Baumgartner (✉)[1] · V.P. Pastushenko
Institute for Biophysics, University of Linz, 4040 Linz, Austria

Present address:
[1] Institute of Anatomy, University of Würzburg,
97070 Würzburg, Germany
e-mail: werner.baumgartner@mail.uni-wuerzburg.de

surrounding electrolyte solution characterized by the parameter of the Debye-Hückel theory κ (the reciprocal of the characteristic Debye length), which reads:

$$\kappa = \sqrt{\frac{2IF^2}{RT\varepsilon}}$$

Here F is the Faraday number, RT is the gas constant times the absolute temperature, ε is the dielectric permittivity of the electrolyte solution, and I is the ionic strength of the solution defined as $I = \frac{1}{2}\sum_i z_i^2 c_i$ denoting the valence and c_i the concentration of ions of type i. The geometry of the problem is depicted in Fig. 1A. A constant field of strength E is applied in the z-direction which is also the main axis of the molecule. The electrostatic potential measured in units of RT/F and denoted by ψ is described by the Poisson-Boltzmann equation:

$$\nabla^2\psi - \kappa^2\psi = 0 \tag{1}$$

with the boundary conditions

$$-\frac{\partial\psi}{\partial r}\bigg|_{\mathrm{r=a}} = \frac{F\sigma}{RT\varepsilon} \quad \text{and} \quad \psi(\infty) = 0$$

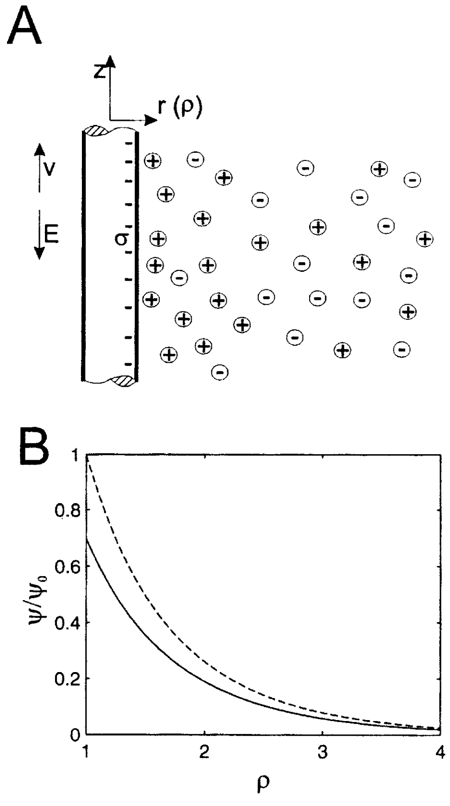

Fig. 1 **A** Geometry of the problem. The molecule is assumed to be stretched and cooriented with the homogenous electric field. The z-axis is the main axis of the molecule. Because of the cylindrical symmetry, only one additional coordinate is required, the radial distance r. The molecule has the radius a and carries a surface charge density σ This induces a potential profile in the electrolyte solution according to Eq. (4) (shown in **B**). Here the electrical potential is normalized to ψ/ψ_0. The *dotted line* shows the potential for the approximation assuming flat geometry. The molecules' radius (in units of the Debye length $1/\kappa$) is assumed $\alpha = 1$

To find a solution for the molecules' velocity, one has to solve the hydrodynamic problem which is described by the Navier-Stokes equation including the force due to the electric field and the friction force due to liquid flow through the gel (Pastushenko and Donath 1976a; Pastushenko 1991a):

$$\underbrace{\eta\nabla^2\upsilon}_{A} - \underbrace{f\upsilon}_{B} - \underbrace{2cFE\psi}_{c} = 0 \tag{2}$$

The term A is due to viscous shear stress in the electrolyte itself. The term C is the electric force acting on a unit volume due to the induced volume charge density in the electrolyte. Term B describes the influence of the gel, with f denoting a volume friction coefficient (Pastushenko and Donath 1976a; Donath and Pastushenko 1980; Pastushenko 1991a). If the buffer flows through the gel with velocity υ, the force $-f\upsilon$ is acting on the unit volume of the buffer owing to the viscous interaction with the gel fibres. As shown previously (Pastushenko 1991b) this coefficient f is exactly the reciprocal of the specific hydrodynamic permeability of the gel. Thus, it is a directly accessible parameter. The boundary conditions for the Navier-Stokes equation are:

$$\upsilon(\infty) = 0 \quad \text{and} \quad \upsilon(\mathrm{a}) = \upsilon_{\mathrm{m}}$$

where υ_{m} is the steady-state velocity of the polymer molecule. This velocity may be found from the balance of the electric force acting on the molecule and the shear force at the surface of the molecule, i.e.

$$\eta\frac{\partial\upsilon}{\partial r}\bigg|_{\mathrm{r=a}} + E\sigma = 0$$

The solution of Eq. (2), using the above boundary conditions, yields the velocity profile near the surface of the molecule. Furthermore, the velocity of the particle corresponds to the liquid velocity at the surface of the molecule.

Potential distribution

For convenience we introduced a dimensionless radial coordinate ρ and the molecules dimensionless radius α as

$$\rho = \kappa r \quad \text{and} \quad \alpha = \kappa \mathrm{a}$$

which allows to rewrite Eq. (1) as

$$\frac{1}{\rho}\frac{\mathrm{d}}{\mathrm{d}\rho}\rho\frac{\mathrm{d}\psi}{\mathrm{d}\rho} - \psi = 0 \tag{3}$$

and the boundary conditions as

$$-\frac{\partial\psi}{\partial\rho}\bigg|_{\rho=\alpha} = \frac{F\sigma}{RT\varepsilon\kappa} \quad \text{and} \quad \psi(\infty) = 0$$

In general, the solution of Eq. (3) is a linear combination of the modified Bessel functions $I_0(\rho)$ and $K_0(\rho)$, respectively {Abramovitz}. Due to the second boundary condition the solution is proportional to $K_0(\rho)$, because $I_0(\rho)$ grows to infinity with growing ρ. The proportionality coefficient results from the first boundary condition:

$$\psi = \psi_0 \frac{K_0(\rho)}{K_1(\alpha)} = \psi_\alpha \frac{K_0(\rho)}{K_1(\alpha)} \tag{4}$$

with $\psi_0 = \kappa\sigma\, 2IF$ denoting the surface potential of a flat surface with the same surface charge density σ, and $\psi_\alpha = \psi(\alpha)$ is the surface potential in the cylindrical geometry. We denote the ratio ψ_α/ψ_0 as

$$R(\alpha) = \frac{K_0(\alpha)}{K_1(\alpha)} \tag{5}$$

According to Eq. (4) the electric field drops radially from the polymer molecule in the form of the modified Bessel function K_0. This also corresponds to the distribution of ions in the buffer surrounding the polymer if $\psi_\alpha \ll 1$.

Liquid flow profile and electrophoretic mobility

For convenience we rewrite the Navier-Stokes equation in dimensionless form introducing the dimensionless parameter

$$\lambda = \frac{\sqrt{f/\eta}}{\kappa}$$

This parameter is the ratio of the Debye length $1/\kappa$ and an "effective pore size"

$$\sqrt{\eta/f}$$

comprising the gel density and the electrolytes' viscosity. Furthermore we introduce the liquid's velocity v measured in units of Smoluchowsky's velocity υ_s, i.e.

$$v = \frac{\upsilon}{\upsilon_s} \quad \text{with} \quad \upsilon_s = \frac{\sigma E}{\eta\kappa}$$

Using these notations the Navier-Stokes equation can be rewritten in cylindrical coordinates as

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial v}{\partial\rho}\right) - \lambda^2 v = \frac{K_0(\rho)}{K_1(\alpha)} \tag{6}$$

with the boundary conditions

$$v(\infty) = 0 \quad \text{and} \quad \left.\frac{\partial v}{\partial\rho}\right|_\alpha = -1 \tag{7}$$

Solving this boundary problem using the method of constants' variation {Zeidler} yields the liquid velocity profile:

$$v(\rho) = \frac{\lambda K_0(\lambda\rho)/K_1(\lambda\alpha) - K_0(\rho)/K_1(\alpha)}{\lambda^2 - 1} \tag{8}$$

This expression for the liquid velocity at a certain radial distance from the molecule depends only on the parameters λ and α. By substituting $\rho = \alpha$, the resulting molecule's velocity is obtained to be:

$$v_m = v(\alpha) = \frac{\lambda R(\lambda\alpha) - R(\alpha)}{\lambda^2 - 1} \tag{9}$$

with $R(x) = K_0(x)/K_1(x)$ as defined in Eq. (5). To eliminate the uncertainty at $\lambda = 1$, de l'Hospitals rule (Zeidler et al. 1996) has to be applied yielding

$$\lim_{\lambda\to 1} v(\rho) = \frac{K_0(\rho)[2 + \alpha R(\alpha)] - \rho K_1(\rho)}{2K_1(\alpha)} \tag{10}$$

$$\lim_{\lambda\to 1} v_m = R(\alpha) + \frac{\alpha}{2}\left[R(\alpha)^2 - 1\right] \tag{11}$$

Obviously for $\lambda = \infty$, which corresponds to an extremely dense gel, $v_m = 0$. In the case of a very diluted gel (pure electrolyte solution) $\lambda \approx 0$, yielding:

$$\lim_{\lambda\to 0} v_m = R(\alpha)$$

Thus the ratio $R(\alpha)$ is simultaneously the ratio of the electrophoretic velocities in cylindrical and flat geometry.

Another special case is that of a large molecular diameter in comparison to the Debye length, i.e. $\alpha \gg 1$. Using the asymptotic expression

$$K_n(z) \approx \sqrt{\pi/(2z)}\,e^{-z}$$

for $z \gg 1$, $n = 0, 1$ (Abramowitz and Stegun 1972), we obtain:

$$\lim_{\alpha\to\infty} v(\rho) = \sqrt{\frac{\alpha}{\rho}}\,\frac{\lambda e^{-\lambda(\rho-\alpha)} - e^{-(\rho-\alpha)}}{\lambda^2 - 1} \quad \text{and} \quad \lim_{\alpha\to\infty} v_m = \frac{1}{1+\lambda}$$

which corresponds to the flat geometry already considered (Pastushenko 1991a). Furthermore, from Eq. (9) we can derive an expression for the electrophoretic mobility which is defined as the molecules' velocity (in standard dimensions) divided by the external electric field strength, E:

$$b = \frac{v_m \upsilon_s}{E} = \frac{\sigma}{\eta\kappa}\,\frac{\lambda R(\lambda\alpha) - R(\alpha)}{\lambda^2 - 1} \tag{12}$$

In the literature the velocity of a molecule is defined by E/ζ (Slater et al. 1987; Sambrook et al. 1989). Here ζ is the initially mentioned phenomenological coefficient, termed viscous friction coefficient. In other words, the electrophoretic mobility is the reciprocal of the viscous friction coefficient.

Results and discussion

We have calculated the steady-state liquid flow velocity profile around a polymer molecule in a gel. Typical profiles are depicted in Fig. 2. The liquid's velocity drops to zero at a finite distance from the molecules' surface and decreases further to negative values before finally approaching zero. This is due to the fact that at

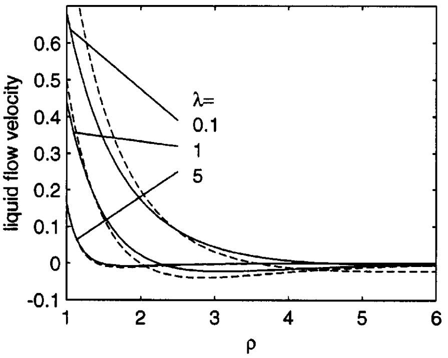

Fig. 2 Some typical velocity profiles for the liquid flow in the vicinity of the polymer molecule. The molecule is assumed to have a radius equal the Debye length, i.e. $\alpha = 1$. The curves are calculated for $\lambda = 0.1$, 1 and 5. The *dotted lines* represent the approximation assuming flat geometry. The non monotonous behavior is explained in the text

finite distance the gel molecules inhibit the liquid flow and thus they act like additional counter ions (for a detailed discussion, see (Pastushenko 1975; 1991a). In the sense of momentum flow the solution is balanced, i.e.

$$\int_{\alpha}^{\infty} v(\rho)\rho \, \mathrm{d}\rho = 0$$

with $v(\rho)$ according to Eq. (8) and (10). This means that there is no net flux of water.

By setting $\rho = \alpha$ we obtained an expression for the molecules' velocity which depends on α and λ. The velocity v_{m} is shown for representative parameters in Fig. 3. As evident from this figure, the theory assuming flat geometry (Pastushenko 1991a) differs remarkably from our results assuming cylindrical geometry for small α and small λ. For DNA, α is about 1 in physiological salt solution (≈100 mM NaCl); thus for dilute gel the deviation of the theory for flat geometry from the theory for cylindrical geometry is about 30%.

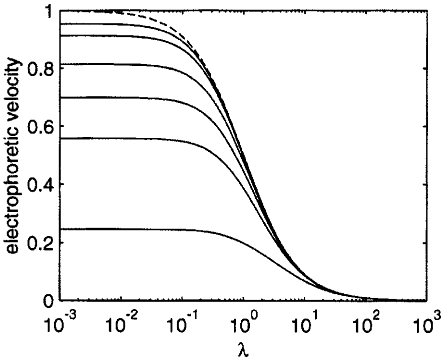

Fig. 3 The electrophoretic velocity v_{m} (in units of Smoluchowsky's velocity) of the polymer molecule in dependence on the molecules' radius (in units of the Debye length) α and the parameter λ. The curves are calculated for (*upwards*) $\alpha = 0.1$, 0.5, 1, 2, 5, 10 and ∞ (*dotted line*) according to Eq. (9). In loose gels ($\lambda \lessapprox 1$) the deviation from the approximation assuming flat geometry ($\alpha = \infty$, *dotted line*) increases with decreasing α as expected; for dense gels ($\lambda \gtrapprox 10$) the value of α has smaller effects

The expressions derived above do not use phenomenological coefficients with exception of the volume friction coefficient, which is a measure for the gel density. This volume friction coefficient is the reciprocal of the hydrodynamic permeability of the gel, which can be measured directly. Thus the influence of ion strength, viscosity, charge, electric field strength, and gel density can be analyzed directly.

The advantage of the dimensionless derivation presented here is that the results depend only on the two parameters α and λ. Thus calculating these two parameters for any given experimental arrangement allows us to find a solution just from a transformation of the results in Fig. 3 by similarity theory.

The derived theory is based on the assumption of negligible interaction of the polymer molecule with the gel fibers. This assumption, chosen for theoretical simplicity, allows us to analyze the indirect influence of the gel fibers owing to hydrodynamic coupling during gel electrophoresis.

Furthermore, the results described here are based on stretched molecules with negligible end effects and gel conditions with uncharged gel fibres and for steady and homogenous electric fields. However, the formulas derived here can be taken for generalization as already exemplified in the literature for PFGE (e.g. Cantor et al. 1988; Pastushenko 1991b). These generalizations are models for the reorientation of the polymer molecules after a change in the direction of the field, for direct steric coupling of the polymer and the gel fibers, for the influence of charged gel fibres, and for the effective length of the molecule due to entropic reasons (very long molecules will not be ideally stretched). If these models can be combined with the theory derived here, it will allow us to investigate the influence of the above-mentioned parameters (ionic strength, charge, gel density, and field strength) for a variety of interesting applications of gel electrophoresis. Thus by a deeper understanding of PFGE it may be possible to further optimize this important biochemical tool for sophisticated analytical and preparative applications.

Acknowledgements We are grateful to Profs. D. Drenckhahn, A.M. Baumgartner and A.V. Pastushenko for helpful discussions. The Austrian Research Funds (project S6607) and the Deutsche Forschungsgemeinschaft (SFB 176 and SFB 487) are acknowledged for financial support.

References

Abramovitz M, Stegun IA (1972) Handbook of mathematical functions, with formulas, graphs and tables. Dover, New York

Cantor CR, Smith CL, Mathew MK (1988) Pulsed-field gel electrophoresis of very large DNA molecules. Annu Rev Biophys Biophys Chem 17: 287–304

Donath E, Pastushenko VP (1980) Bioelectrochem Bioenerget 7: 31–40

Lumpkin OJ, Déjardin R, Zimm BH (1985) Theory of gel electrophoresis of DNA. Biopolymers 24: 1573–1593

Pastushenko VP (1975) Theory of selfelectrophoretic mechanism of bacterial movement. Bioelectrochem Bioenerget 2: 52–60

Pastushenko VP (1991a) Mobility and electrophoretic mobility of long linear polymer molecules in gels. Appl Theor Electrophoresis 1: 313–316

Pastushenko VP (1991b) Tension of DNA molecules and angular dependence of PFGE efficiency: a theoretical analysis. Appl Theor Electrophoresis 1: 317–321

Pastushenko VP, Donath E (1976a) On the electrophoretic mobility of cells coated with charged glycoprotein layer. Studia Biophys 56: 7–8

Pastushenko VP, Donath E (1976b) Potential distribution near the cell coated with charged glycoprotein layer. Studia Biophys 56: 9–10

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. A laboratory manual, vol 1. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Slater GW, Rousseau J, Noolandi J (1987) On the stretching of DNA in the reptation theories of gel electrophoresis. Biopolymers 26: 863–872

Zeidler E, Schwarz R, Hackbusch W (1996) Teubner Taschenbuch der Mathematik. Teubner, Stuttgart