Influence of Agarose Concentration and Electric Field Strength on the Separation of DNA-Fragments During Electrophoresis

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Introduction

Gel electrophoretic separation of DNA fragments has been used extensively in forensic DNA typing. DNAs mobility in gels depends upon fragment length and some other features(Fisher and Dingman 1971). But the mechanism of gel electrophoresis is not well understood. The migration of the DNA molecules through the pores of the matrix must play an important role in molecular weight separations, since the electrophoretic mobility of DNA in free solution is independent of molecular weight(Olivera et al. 1964). The most successful theory of electrophoresis is the reptation theory of deGennes(1971) and Doi & Edwards(1978). The reptation theory describes the migration of polymers in the presence of fixed obstacles. The DNA fragment move along its axis through a "tube" in a neutral gel under the influence of an electric field(Lumpkin et al. 1985). The apparent mobility of DNA fragments (0.5-12kb) decreases with decreasing electric field strength and with increasing gel concentration(Nancy 1985). Separation capacity for DNA fragments is the distance between two fragments in gel electrophoresis.

Materials and Methods

The sample DNA used for the electrophoresis experiments was the standard marker Lambda Hind III(Boehringer), which contained DNA fragments of 0.564, 2.027, 2.322, 4.361, 6.557, 9.416 and 23.13kb. The loading mass of DNA was 1.2µg per well. The agarose used for all studies was Ultrapure Agarose (Gibco BRL, electroendosmosis=0.10-0.15). All gels were cast and run in Tris-borate buffer(TBE: 0.089M boric acid, 0.089M Tris-base, and 0.002M EDTA, pH8.0). The agarose was dissolved by boiling on a magnet hot plat, lost water was replaced by distilled water. After pouring, the gels were allowed to solidify for 40 min, after which the comb was removed, the gels were flooded with buffer in electrophoresis chamber(GNA-200, Pharmacia) and were allowed to stand for 20 min. Six gels with different agarose concentrations were poured on the same gel supporter. Then the DNA samples were applied and electrophoresis was started. In a preliminary investigation we have found that DNA fragments migrate with a constant velocity when the other conditions (agarose concentration, electric field strength, etc.) are constant. All electrophoresis were run at 20°C(regulated with Desaga Frigostat, Desaga). The electric field was supplied and controlled with 2197 Power Supply(LKB Bromma). After the electrophoresis the gels were stained with ethidium bromide(5µg/ml) for 15 min, and then were photographed under UV

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light. The migration distances(S, mm) of the DNA fragments were measured and the velocity was calculated as follows:

$$v=S/t \qquad \text{(Eq.1)}$$

Here t was the electrophoresis time(hours). The migration distance of the 0.564 kilobase fragment(velocity was v_1, mm/h) was converted to 180 mm. The relative migration distance(S_r, mm) of a DNA fragment (velocity was v, mm/h) was calculated with the following Equation:

$$S_r=180xv/v_1 \qquad \text{(Eq.2)}$$

Results and Discussion

1. *Separation capacity of gels with different agarose concentration.* The relationship between log(kb) and the relative migration distance of the DNA fragments in gels with different agarose concen-trations(1.6%, 1.4%, 1.2%, 1.0%, 0.8% and 0.6%) for 4V/cm electric field streng is illustrated in Figure I. The smaller ones of the DNA fragments over 4.361kb show a rapid reduction of the relative migration distance with increasing agarose concentration. This causes a decrease of the distance (separation capacity) between these fragments. The longer ones of the fragments between 0.564-2.027kb also show a rapid reduction of the relative migration distance, so that the distances(separation capacity) between these fragments increase with increasing agarose concentration. For the fragment length range from 2.027 and 4.361kb there is no differnce of the separation capacity between 1% and 0.8% agarose gels. This is an important result for forensic DNA-typing, considering the rehybridisation of blots with polymorphic probes revealing different allele size distributions(e.g. MS43/Hinf I 3.5-16kb and YNH24/Hinf I 2.1-5.5kb). Since 1% agarose gels show a decreased separation capacity for fragments longer than 4.361kb and smaller fragments are equally well separated in 0.8% gels, an agarose concentration of 0.8% should be used.

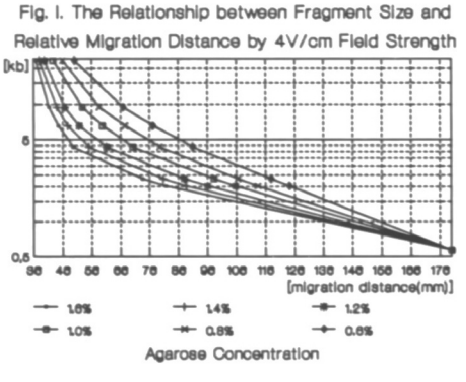
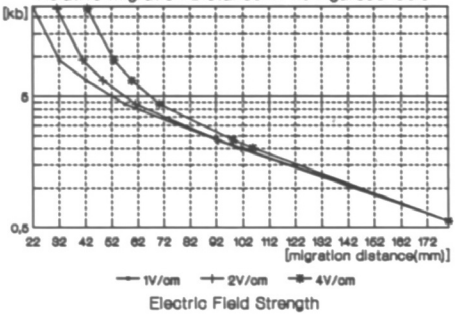


Fig. I. The Relationship between Fragment Size and Relative Migration Distance by 4V/cm Field Strength

2. *Separation capacity of different electric field strength.* Figure II shows the relationship between the log(kb) and the relative migration distance of DNA fragments in 1.0% agarose concentration for different electric field strength. The relative migration distances of the longer fragments increase more rapidly with increasing electric field strength than those of the smaller ones. Therefore the distances between the fragments(especially the fragments longer than 4.361kb) are reduced with increasing electric field strength. This reduction is particularly evident from 2V/cm to 4V/cm. For the fragments between 0.564kb and 2.027kb the relative migration distance does not increase as rapidly as

for the fragments between 2.027kb and 23.13kb with increasing electric field strength. The migration of long fragments(over 2kb) through agarose gels is more dependent upon the field strength than on molecular weight. This is probably caused by the field-induced distortion (Lumpkin et al. 1985)of the long fragments by high electric field strength. According to our investigation, 4V/cm electric field strength can be employed for the separation of DNA fragments smaller than 2kb, without much loss of separation capacity. Moreover, only about 1/4 of the electrophoresis time for 1V/cm is needed. But since for fragments longer than 2kb the separation capacity of high voltage electric fields is much lower, a low electric field strength(e.g. 1V/cm) should be used.

Fig. 11. The Relationship between Fragment Size and Relative Migration Distance in 1% Agarose Gels



Summary

The migration distances of DNA fragments undergoing gel electrophoresis were compared, in order to study the separation capacity of gels with different agarose concentrations and of different electric field strength for given DNA fragments. High electric field strengths have lower separation capacity for DNA fragments over 2kb. A rapid separation of DNA fragments with high voltage electric field strength is not to recommend. To the separation of DNA fragments under 2kb electric field strength has scarcely influence. For DNA fragments of 4-23kb a low agarose concentration has a higher separation capacity. The fragments between 2 and 0.5kb can be separated better in gels with higher agarose concentration. Since the fragment length range from 2-4kb is equally well separated in 0.8% and 1% agarose gels, 0.8% agarose concentration is suggested for forensic DNA-typing of Hinf I digests.

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