TECHNICAL DEVELOPMENT

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INEXPENSIVE METHOD OF DNA GEL ELECTROPHORESIS

NAPASRI TALUPPETH, CHEEPTIP BENYAJATI* and NONGNUJ TANPHAICHITR

Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 4, Thailand

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Summary

Agar, which is much less expensive than agarose, was used in place of agarose in DNA gel electrophoresis. Our results clearly indicate that they are both equally good for separating DNA fragments of different sizes. In addition, we have devised an inexpensive "dark box" for taking photographs of fluorescing DNA bands on this gel. This equipment is much less expensive than the cost of setting up a dark room for this purpose.

Introduction

Agar and agarose have long been used in electrophoresis of RNA[1-5]. β-D-galactopyranose and 3,6-anhydro-L-galactose form the repeating units both in agar and agarose, although the former also contains pectin[6]. However, several investigators have claimed that agar does not give as sharp resolution as agarose in separating RNA macromolecules of different sizes, due to the adsorption effect of proteins in agar to RNA, making the mobility of RNA slower than that in agarose[4,5]. Consequently, this may lead to diffusion of RNA bands.

Recently, studies on the repeating units of DNA on chromatin have become very popular, and agarose gel or composite of low per cent acrylamide and agarose have been used to separate these repeating units[7-11]. The selection of agarose has been also very specific and only the products from a few companies such as

*Present address: The Johns Hopkins University Mergenthaler Laboratory, Baltimore, MD. 21218, USA.

those from BDH (British Drug House) or L'Industrie Biological Francaise and Marine Colloids (Seakem agarose) are recommended[6, 9].

TABLE I: PRICE PER 100 g OF AGAROSE AND AGAR FROM DIFFERENT COMPANIES

Type	Price
BDH agarose	US$ 88 (Baht 1,750)[a]
Aldrich agarose	US$ 180 (Baht 3,600)[b]
Fisher (Seakem) agarose	US$ 241 (Baht 4,820)[b]
Seriwat agar	US$ 1.80 (Baht 36)

[a]Purchased from a local agent
[b]Price included shipping

Unfortunately, agarose specially prepared for electrophoresis is very expensive (Table I) as compared to agar. In view of this and also of abundance of agar in Asia, we have tested the possibility of replacing agarose with agar in DNA gel electrophoresis. Our results shown here indicate that both agar and agarose are equally good for this purpose.

In addition, we demonstrate our inexpensive home-made apparatus for taking photographs of DNA bands fluorescing under ultraviolet light after being inserted with ethidium bromide.

Materials and Methods

Agarose was purchased from BDH, Fisher Chemical Company (distributor of Seakem agarose) and Aldrich Company. Agar was obtained from local supermarkets. Three kinds of agar were used: ground agar powder of Seriwat Food Company, Japanese fibrous agar, and mixture of ground agar and sugar of Chang Bin Brand. Other Chemicals were of reagent grade.

The "dark box" of which dimension is shown in Fig. 2 was constructed by Yun Yong Plastic Shop, 22nd July Circle Bangkok, Thailand at the price of $20 (Baht 400).

DNA repeating units of rat testis were prepared by digesting rat testis nuclei with micrococcal nuclease (Worthington) at 150 units/mg of DNA at 37°C[10]. Sizes of testicular DNA repeating units were determined using the known sizes of those of rat liver nuclei[11]. This analysis yielded the size of testicular nucleosomal DNA of around 200 nucleotide base pairs.

Agarose and agar gel (1.2%) was separately prepared in the vertical slab plates (15 × 13 × 0.2 cm^3) as described by Akroyd[12]. For agarose and ground agar, the desired weight of the powder was stirred in aqueous buffer and heated to 50-60°C. When the material was completely dissolved, the solution was directly poured into the plates. On the other hand, Japanese fibrous agar always formed the undesired particulate and had to be filtered away through warm filter paper before use.

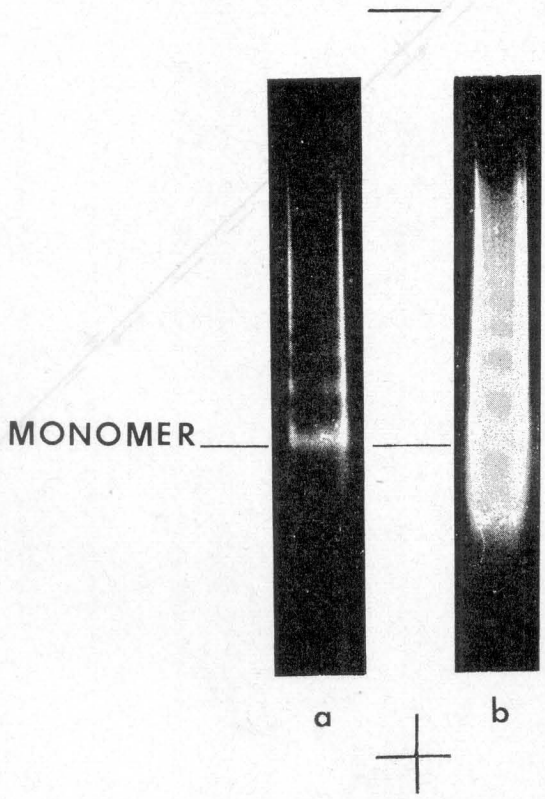


Fig. 1. DNA fragments of rat testis obtained after the nuclei were digested with micrococcal nuclease (150 units/mg of DNA) for 30 min. Fragments of multiple core units were separated on Seriwat agar (a) and BDH agarose gel (b) (1.2%) with the buffer system of Hayward and Smith[12] as described in Materials and Methods.

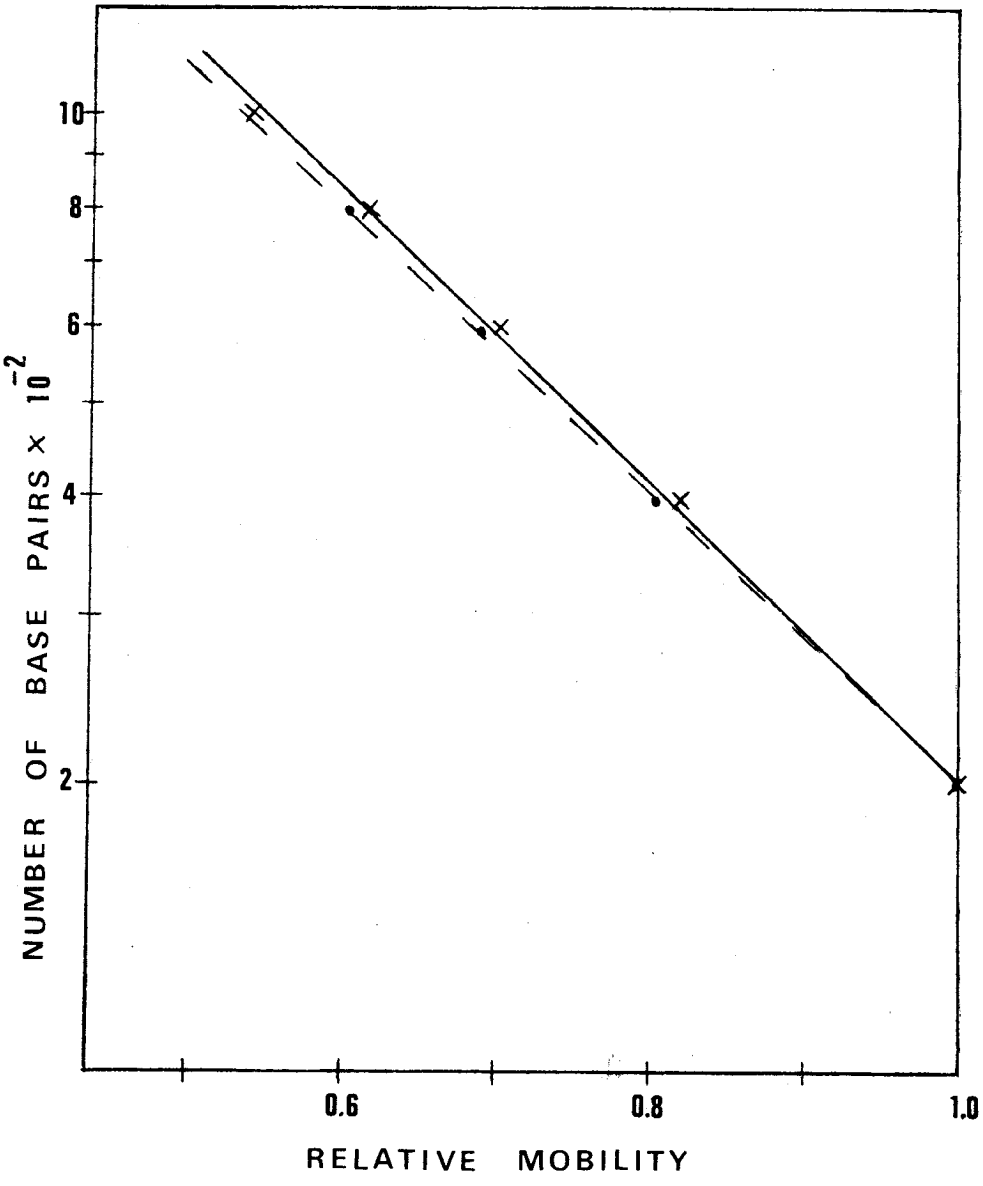
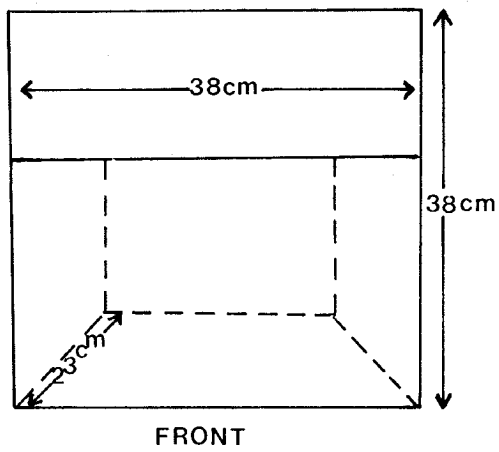
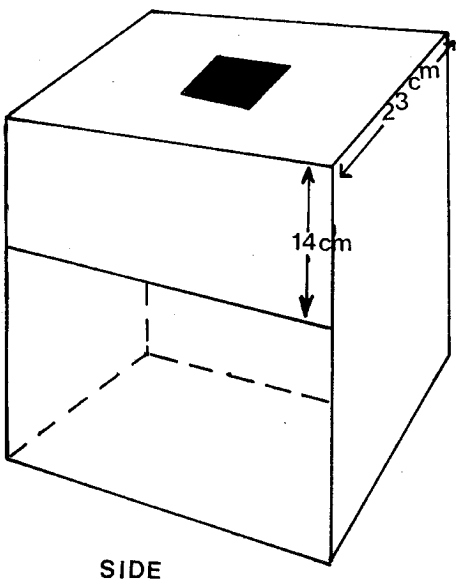


Fig. 2. Plot of logarithm of number of base pairs of testicular DNA fragments prepared as in Fig. 1 versus relative mobility. The number of base pairs of these DNA fragments were determined by comparing them to the known base pairs of rat liver DNA after being digested by endogenous endonuclease[13]. – – – – – – – is the plot obtained from Seriwat agar gel, whereas ——————— from BDH agarose gel.





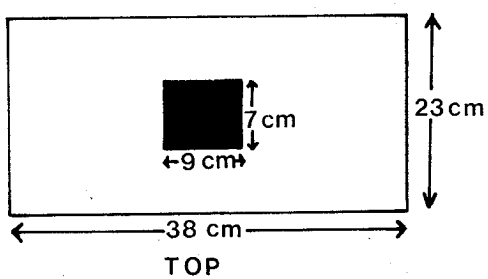


Fig. 3. Drawings of dimension of the dark box used for taking photographs of fluorescing DNA bands under ultraviolet light. DNA gel was placed inside the box between two ultraviolet lamps. The half open front side was covered with dark fabric and the camera was placed over the hole on the top (shown as the dark area on the top).

The buffer system for electrophoresis was of Hayward and Smith (0.01 M Tris base, 0.001 M EDTA and 0.01 M sodium acetate, pH 7.8[13]. Electrophoresis was done at 150 volt at 4°C until bromophenol blue was 2 cm from the bottom end.

Usually, 0.03% of ethidium bromide was also included in the gel. Alternatively, DNA bands were stained with 0.002% ethidium bromide solution after electrophoresis. Photographs of DNA gel were taken under ultraviolet light in our "home-made" dark box, using Olympus Camera Model OM-1 with a red filter. The camera aperture was set at 5.6 and let open for 20, 40 and 60 s, when Tri-X ASA 400 film was used.

Results and Discussion

It is clear from Fig. 1 that agar from Seriwat Food Store is as good as agarose from BDH in separating DNA repeating units of rat testis nuclei. Nonetheless, DNA-ethidium bromide complex fluoresces more in BDH agarose. The reason for this is unclear. It is possiple that BDH agarose contains other contaminants which reacts with ethidium bromide, as the background is relatively more fluorescent. Agarose from Fisher and Aldrich also give similar results (unpublished). The running time for Seriwat agar and agarose gel is not significantly different. However, when Japanese fibrous agar or Chang Bin agar were used, the running time was usually long and the separation was undesirable, most likely due to the diffusion of DNA samples (unpublished).

When DNA relative mobilities both in agarose gel and Seriwat agar gel were plotted against logarithm of number of DNA base pairs, a linear relationship was obtained. The two lines are not significantly different. This explicitly suggests that the electrophoresis of DNA in Seriwat agar gel is not affected by electroosmosis or other aberrant factors. Besides this inexpensive price of agar, it is also much less fragile than agarose and the agar slab can be easily and conveniently handled.

Several ways to identify DNA bands after electrophoresis have been described. For example, they can be scanned directly at 260 nm. Although this is suitable for quantitative analysis, it is inconvenient when simultaneous comparison of several samples is needed. Alternatively, DNA bands are stained with dyes such as toluidine blue, methylene blue, or pyronin Y[6]. However, destaining of these dyes takes several hours. More preferentially, DNA double strands are inserted with ethidium bromide (0.002-0.005%) which can fluoresce in the ultraviolet light and photographs can be taken[6]. DNA shows in the negatives as dark bands, and if sizable films are used (e.g. sheet films of $4\frac{1}{4} \times 5$ cm or $3\frac{1}{2} \times 4$ cm). The negatives of each sample can directly be scanned in the gel scanner using any wavelength.

In order to get good contrasting bands of fluorescing DNA, photographs need to be taken in the dark. In view of expensive cost of setting up a dark room, we have devised a dark box as shown in Fig. 3. Photographing these DNA gels inside this dark box with dark fabric over the open side and any regular camera on the top hole gives satisfactory results, even this is carried out in the open daylight laboratory (Fig. 1).

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บทคัดย่อ

วุ้นอาการ์ของเสรีวัฒน์ ได้ถูกนำมาใช้แทนวุ้นอาการ์โรสเกรดดีในการแยกกรดดีออกซีไรโบนิวเคลอิคด้วยไฟฟ้า ผลการทดลองของเราบ่งชัดว่าวุ้นทั้งสองชนิดมีประสิทธิภาพดีเท่ากันในการแยกนี้ วุ้นอาการ์ราคาถูกกว่าวุ้นอาการ์โรสกว่า 100 เท่าตัว การใช้วุ้นอาการ์แทนอาการ์โรสจะช่วยประหยัดค่าใช้จ่ายในการทดลองทางวิทยาศาสตร์มากขึ้น

อนึ่ง เราได้ประดิษฐ์เครื่องมือ "กล่องมืด" ซึ่งสามารถใช้แทนห้องมืดในการถ่ายรูปของกรดดีออกซี-นิวเคลอิคซึ่งเปล่งแสงฟลูออเรสเซนส์ในรังสีอุลตราไวโอเลตหลังจากถูกแทรกเคียงด้วยอิธิเดียมโบรไมด์