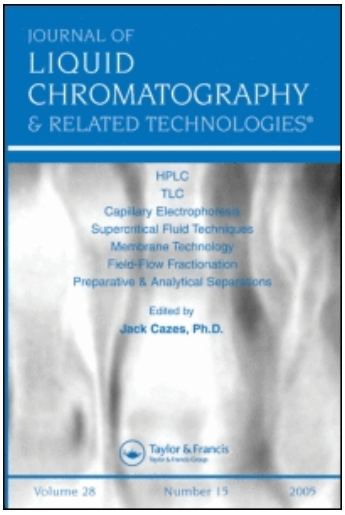

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Three-Dimensional Electropherogram for the Separation of Oligodeoxy-Nucleotides and DNA Restriction Fragments Using Capillary Gel Electrophoresis with a Photodiode Array Detector

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THREE-DIMENSIONAL ELECTROPHEROGRAM FOR THE SEPARATION OF OLIGODEOXY-NUCLEOTIDES AND DNA RESTRICTION FRAGMENTS USING CAPILLARY GEL ELECTROPHORESIS WITH A PHOTODIODE ARRAY DETECTOR

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ABSTRACT

A photodiode array detection system was applied to capillary gel electrophoresis and gave reliable spectra of single-stranded oligodeoxynucleotides and double-stranded DNA restriction fragments even in the presence of high UV background caused by polyacrylamide within the capillary. Photodiode array detection enabled accurate characterization of separated DNA fragments and was a powerful tool for the identification of oligodeoxynuelcotides.

INTRODUCTION

Slab gel electrophoresis has been widely used as a standard method for the separation of single-stranded DNA and DNA restriction fragments[1,2]. More recently, capillary gel electrophoresis (CGE) is developing rapidly as a powerful new analytical technique for the separation of single- and double-stranded DNA[3-7].

CGE is the automated technique offering the benefits of rapid separation and high resolution in comparison with time-consuming and labor-intensive slab gel electrophoresis.

CGE has some other advantages inherently, e.g., many valuable detectors developed for HPLC will be applicable to CGE. A photodiode array detector has been recognized as a powerful tool for HPLC[8] and capillary zone electrophoresis[9], but it has entered the CGE field more slowly. Due to high UV absorbance of polyacrylamide[10], the investigators may hesitate to apply the photodiode array detector to CGE.

In this paper, we demonstrates that a photodiode array detector is particularly useful for the analysis of spectra for DNA fragments in the presence of high UV background caused by polyacrylamide and the identification of oligodeoxynucleotides.

EXPERIMENTAL

The DNA restriction fragments of a ϕX174DNA/*Hae* III digest (0.24 μg/μL) was purchased from Toyobo (Osaka, Japan). The ϕX174DNA/*Hae* III digest contained 11 fragments of 72, 118, 194, 234, 271, 281, 310, 603, 872, 1078, and 1357 base pairs (bp). The DNA samples were 10-fold diluted with Milli-Q water and stored at -18 °C until use. Oligodeoxynucleotide was purchased from Pharmacia (Uppsala, Sweden). The oligodeoxynucleotide sample was prepared by dissolution of 2.5 units sample into 100 μL Milli-Q water and stored at -18 °C until use. All other chemicals were of analytical-reagent or electrophoretic grade from Wako (Osaka, Japan).

Capillary gel electrophoretic separations were carried out by using an Otsuka CAPI-3000 (Otsuka Electronics, Osaka, Japan) and an HP ^{3D}CE (Yokokawa Analytical Systems, Tokyo, Japan) capillary electrophoresis instruments both equipped with a photodiode array detector. Polyimide coated fused silica capillaries (375 μm o.d., 100 μm i.d., GL Sciences, Tokyo, Japan) of 37.8 cm effective length and 50 cm total length were used. Gel-filled capillaries, in which crosslinked polyacrylamide gel (3 %T and 0.5 %C) or linear polyacrylamide (8 %T and 0 %C) is chemically bound to the capillary wall, were prepared

according to the literature[6,7]. A sample solution was introduced electrophoretically at negative polarity of 10 kV for 5 s into the gel-filled capillary and separated with a running buffer solution (50 mM tris-borate and 1 mM EDTA, pH 8.4) at negative polarity of 10 kV (200 V/cm, 8-15 μA) at 25 °C.

RESULTS AND DISCUSSION

Capillary gel electrophoresis (CGE) shows high resolving power in the single base resolution of single-stranded DNA[3-5] and separates completely a mixture of oligodeoxynucleotides of equal chain lengths but of different base composition[11,12]. In this case, each oligodeoxynucleotide is expected to be identified from its spectrum obtained by using a photodiode array detector, because the wavelength and the molar absorption coefficient for the UV absorption maxima of nucleotides in aqueous solutions are different from each other as follows: deoxyadenosine (260 nm, 1.52 x 10^4), deoxyguanosine (254 nm, 1.30 x 10^4), thymidine (267 nm, 9.65 x 10^3), and deoxycytidine (271 nm, 9.0 x 10^3), respectively[13]. The identification from the spectrum would be much easier and consume less amount of DNA sample than the conventional identification by spiking each peak with authentic sample. Although the high absorbance of polyacrylamide (3% T polyacrylamide gives 2.4-3.0 absorbance unit in the UV range of 190-260 nm and its spectrum is shown in Fig. 3 of ref. 10) within the capillary seems to interfere with reliable measurement for the spectrum of DNA fragment, the CAPI-3000 or the HP ^{3D}CE system, which measures a reference spectrum of polyacrylamide before electrophoresis, stores it on a hard disk, and corrects the background during electrophoresis, allows us to measure the spectrum accurately and obtain fine three-dimensional electropherogram. Other less-absorbing polymers such as dextran are easily employed instead of polyacrylamide.

Figure 1 shows the three-dimensional electropherogram (time-wavelength-absorbance) obtained by the photodiode array detector for the separation of a mixture of single-stranded oligodeoxynucleotides, using linear polyacrylamide (8 %T and 0 %C) filled capillary. We chose 20-mers of deoxyadenylate (dA_{20}) and thymidylate (dT_{20}) as model substrates. All spectrophotometric information over 200-400 nm of the DNA fragment migrating through a on-column cell of the gel-

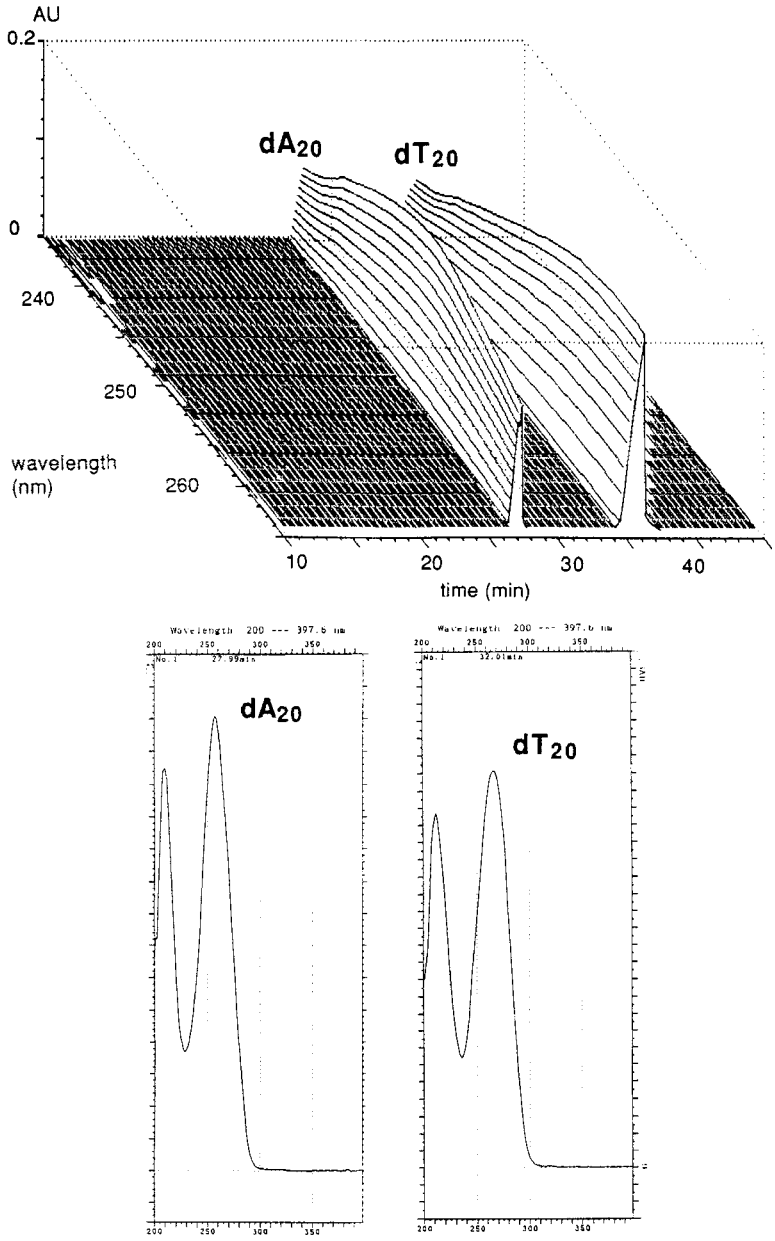

FIGURE. 1 Three-dimensional CGE profile for a mixture of oligodeoxynucleotides, dA_{20} and dT_{20}, obtained by a photodiode array detector, using linear polyacrylamide (8 %T and 0 %C) filled capillary. Conditions: Capillary, 100 µm i.d., 375 µm o.d., 50 cm length, 37.8 cm effective length; running buffer, 50 mM Tris-borate and 1 mM EDTA, pH 8.3; field, 200 V/cm; current, 10 µA; injection, 10 kV for 5 s; capillary temperature, 25 °C; recording, 0.1 AUFS, 230-280 nm.

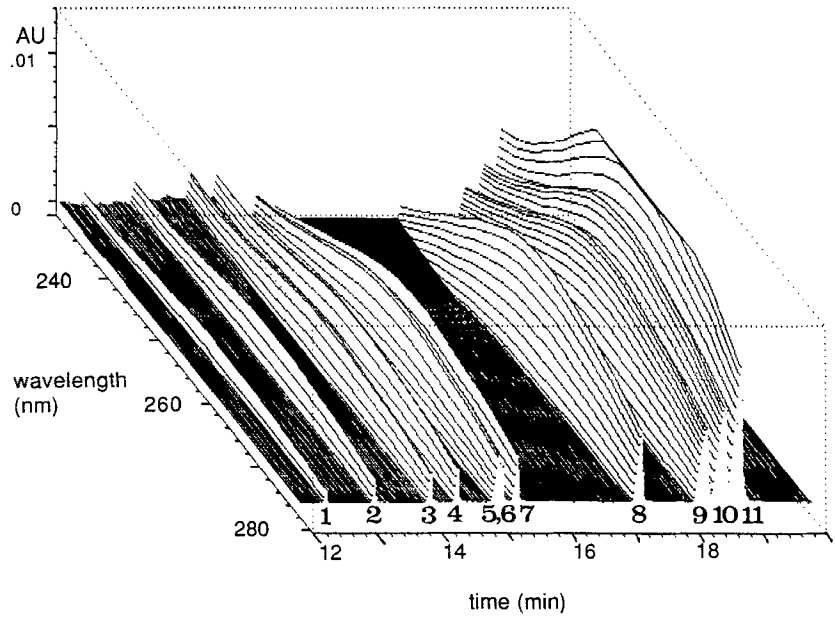

FIGURE. 2 Three-dimensional CGE profile for the *Hae* III restriction digest of ϕX174 DNA, using crosslinked polyacrylamide gel (3 %T and 0.5 %C) filled capillary. Other conditions as in Fig. 1. Peak assignment; 1=72, 2=118, 3=194, 4=234, 5=271, 6=281, 7=310, 8=603, 9=872, 10=1078, and 11=1357 bp.

filled capillary was computer-stored and a part of spectrum was reproduced three-dimensionally. Each well-resolved component is migrated at 27.99 min and 32.01 min, and gives the different absorption spectrum. The spectra of both components, superimposed in Fig. 1, show characteristic absorption maxima of 259 nm for the peak at 27.99 min and 267 nm for the peak at 32.01 min. These peaks, therefore, are assigned to dA_{20} for the former peak and dT_{20} for the latter peak from the spectrophotometric data obtained by the photodiode array detector and the assignments agreeing with those by spiking the authentic samples. These results show that the well-designed photodiode array detector is applicable to obtain the reliable spectrum for the identification of the sample solute migrated into the capillary filled with polyacrylamide having a very high UV absorbance.

We next examine the application of the photodiode array detector to gain three-dimensional electropherogram (Fig. 2) for the separation of a mixture of

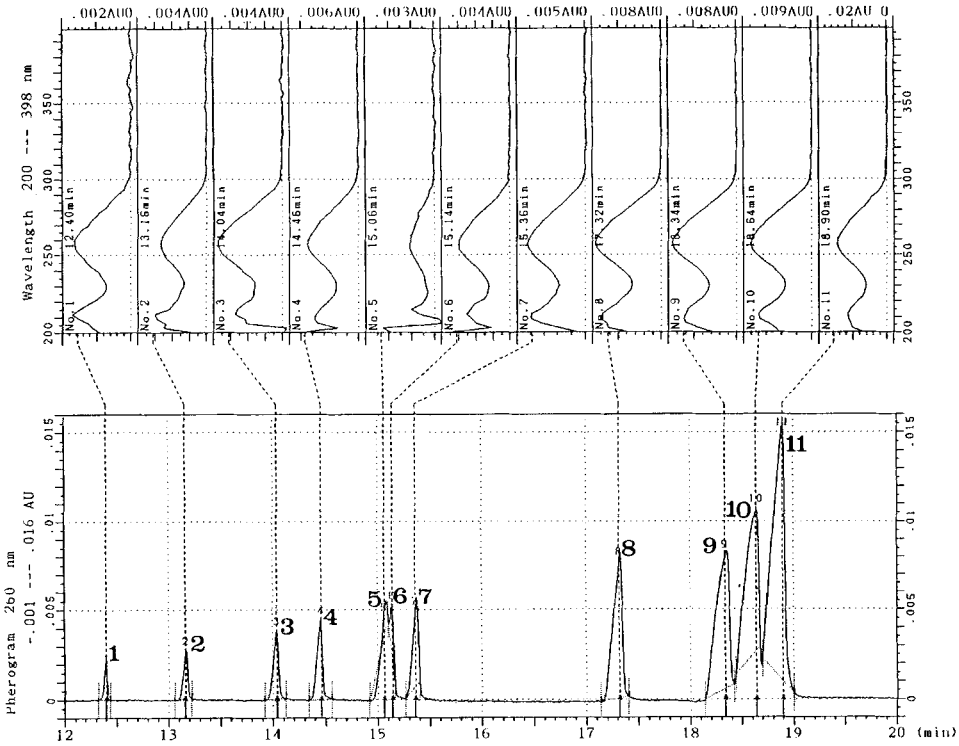

FIGURE. 3 Two-dimensional CGE profile at 260 nm for the *Hae* III restriction digest of ϕX174 DNA and the UV absorption spectrum of each peak. Other conditions as in Fig. 2. Peak assignment; 1=72, 2=118, 3=194, 4=234, 5=271, 6=281, 7=310, 8=603, 9=872, 10=1078, and 11=1357 bp.

double-stranded DNA restriction fragments (ϕX174 DNA/*Hae* III digest), using crosslinked polyacrylamide gel (3 %T and 0.5 %C) filled capillary. All fragments are well resolved and give the similar UV absorption spectrum, having a maximum around 255-260 nm. This illustrates that the photodiode array detector produces easily the three-dimensional electropherogram for the broader base pair range of DNA restriction fragments, ranging from 72 to 1353.

To investigate the feature of these spectra in more detail, two-dimensional electropherogram at specific wavelength (260 nm) and the spectrum of each component was reproduced from the spectrophotometric information stored on the

hard disk as shown in Fig. 3. The electropherogram indicates that the peaks are detected at the AU range from 0.0017 (72 bp) to 0.015 (1353 bp). The wavelength of the absorption maximum can be calculated from the spectrum accurately and it is noteworthy that each spectra of the individual fragments are reliably yielded even from the peak (72 bp) having very low absorbance unit of 0.0017. Each spectrum shows the specific absorption maxima in the range from 255 to 260 nm. These spectra indicate that the measurement of DNA restriction fragments at wavelength range from 250 to 265 nm is recommended for the sensitive detection of each DNA fragment, when a conventional variable-wavelength detector is used for routine analysis. The sensitivities relative to that at 260 nm are approximately 100 (260 nm), 90 (250 nm), and 90 (270 nm), respectively.

These results show that the photodiode array detector is effective for the check of PCR reaction through the identification of PCR product and reactants, including large DNA fragments (100-10,000 bp), oligodeoxynucleotide primer, and deoxynucleoside triphosphates (dNTP). The spectrum of large DNA fragments produced by PCR reaction is easily distinguishable from the spectra of oligodeoxynucleotides and dNTP, if migration time of PCR product is very similar to those of primers and dNTPs.

In this study, we demonstrate that the photodiode array detector is effective for the analysis of spectrum of single- and double-stranded DNA fragments even in the presence of high UV background caused by polyacrylamide within the capillary. The results in Figs. 1-3 clearly illustrate that the photodiode array detector shows very high detectability for the measurement of the absorption maximum from the spectrum of DNA fragments and will be applicable to identify oligodeoxynucleotides.

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