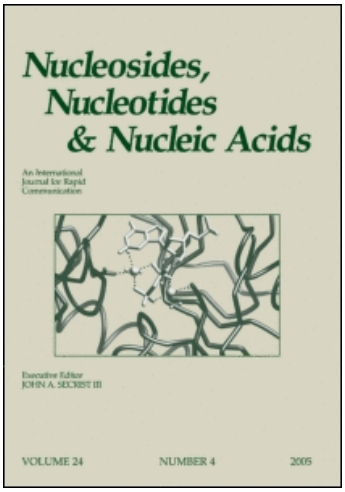

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Evaluation of Capillary HPLC/Mass Spectrometry as an Alternative Analysis Method for Gel Electrophoresis of Oligonucleotides

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ABSTRACT

A method has been developed to monitor the enzymatic incorporation of nucleotides in DNA by electrospray HPLC mass spectrometry. The main advantages of mass spectrometry over electrophoresis are the ability to directly characterize the reaction products and the shorter analysis time.

Key Words: LC/MS; Oligonucleotides; DNA polymers.

INTRODUCTION

Polyacrylamide gel electrophoresis (PAGE) is a commonly used method for analysis of oligonucleotides. Disadvantages are the need of radioactive labeling, the time-consuming development of the gels and the need for reference compounds to be included in each run. In this work the DNA polymerase catalyzed incorporation

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of nucleotide triphosphates into primer-template DNA duplexes was followed with PAGE and with capillary HPLC/MS.[1]

RESULTS AND DISCUSSION

Enzymatic reactions were carried out using Vent (exo-) DNA polymerase. To the primer-template DNA hybrid (2.5 μM), deoxynucleotide triphosphates (dNTPs, 200 μM) and enzyme (0.02 U/μL) were added. After incubation (10 min. at 55°C), the reactions were quenched by freezing the reaction mixtures. Analysis by PAGE has been described previously.[2] The experimental conditions were optimized in such way that enzymatic reaction mixtures could be injected directly onto the HPLC column. Despite the fact that the current conditions are less sensitive, mass spectrometry proved to be a better choice for determination of the oligonucleotide length and

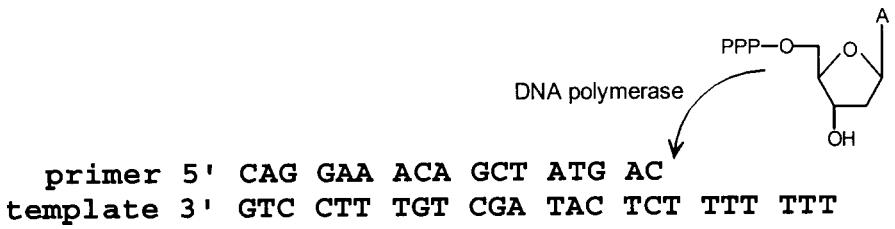

Figure 1. Incorporation of nucleotides using DNA polymerase.

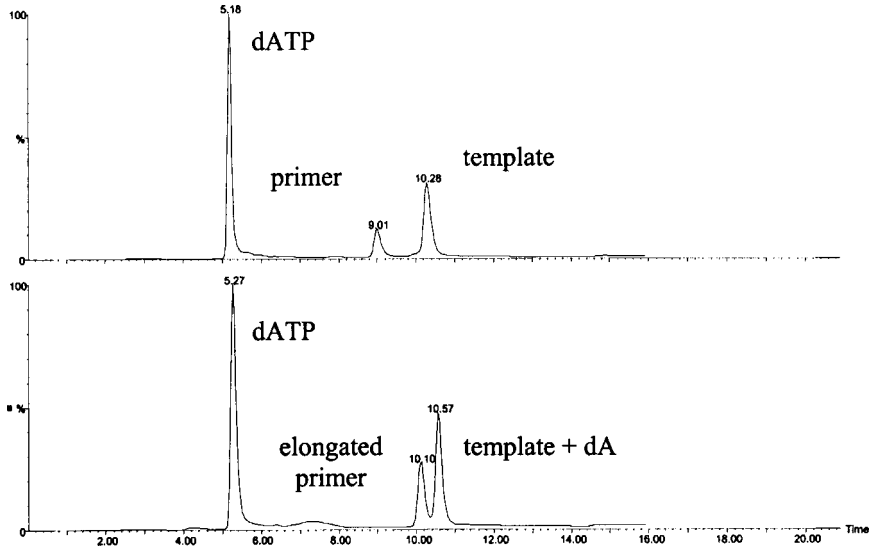

Figure 2. HPLC profile (UV260) of a reaction mixture. Upper panel: DNA duplex + dATP, lower panel: DNA duplex + dATP + enzyme.

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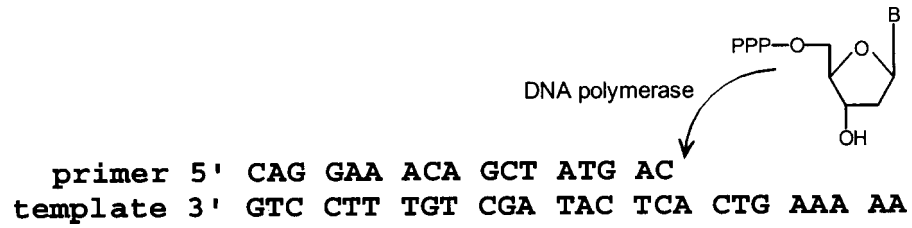

Figure 3. Verification of the sequence specificity by adding a mixture of all four dNTPs.

sequence. Other advantages are the ability for automated analysis and the shorter overall analysis time.

Figure 1 shows the enzymatic incorporation scheme for a single nucleotide triphosphate. Although the primer can be extended by seven residues, we found that at higher enzyme concentrations, an extra nucleotide was appended at the 3′ end of the primer and at the 3′ end of the template. In addition to the UV traces shown in Fig. 2, the electrospray mass spectra obtained in negative ionization mode gave information about the identity of the oligonucleotides in the reaction mixture.

The sequence specificity was verified by adding a mixture of all four dNTPs in the reaction depicted in Fig. 3. The reaction products found were as expected from the template sequence (Fig. 4) and the 3′ end of the primer could be sequenced through fragmentation in the mass spectrometer and proved to be correct.

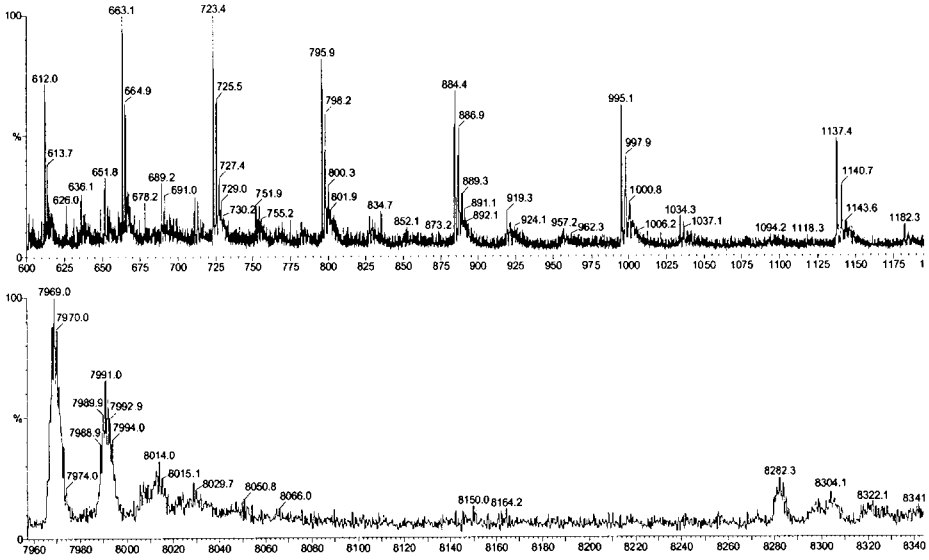

Figure 4. Reaction products formed by enzymatic incorporation as shown in Fig. 3. Lower panel: deconvoluted spectrum showing partial incorporation of an extra nucleotide (mass 8282).

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