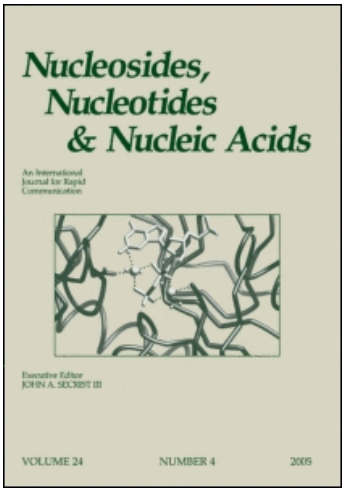

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Development of a New DNA Sequencing Method: 3'-Ester Cleavage Catalyzed by *Taq* DNA Polymerase

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DEVELOPMENT OF A NEW DNA SEQUENCING METHOD : 3'-ESTER CLEAVAGE CATALYZED BY *Taq* DNA POLYMERASE

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ABSTRACT : A new 3'-esterified dTTP is incorporated into DNA by *Taq* DNA polymerase but does not act as a chain terminator. The esterase activity of the polymerase seems to be template dependent and occurs only if the next correct nucleotide is present.

Genetics tests are now widely used to diagnose individuals with genetic disease and to recognize those who are susceptible to disease. DNA testing and research programs such as the Human Genome Project have brought into evidence the need of easy but accurate DNA sequencing methods. Indeed, techniques enabling rapid detection of deletion, insertion or single base pair substitution would be extremely helpful in genetic analysis.

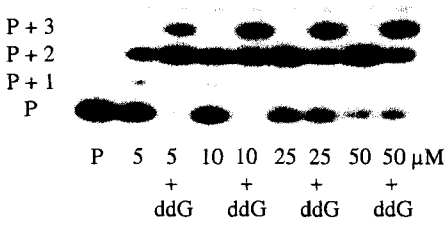
We have recently described a new DNA sequencing method without gel electrophoresis [1]. It consists of enzymatic base specific incorporation of labile terminators at the 3'-OH end of a primer, annealed to a single-strand DNA template. Each nucleotide is characterized by the dye attached to the 3'-protecting group by a convenient treatment will rapidly restaure the 3'-OH end and the liberation of the tag will enable the identification of the incorporated nucleotide. Then the reinitiation of DNA synthesis will be possible.

We have tried to find a common labelling strategy for the four nucleotides occuring in DNA. We have already described a spacer, the 6-*N*-(*N*-methylanthranylamido)-4-oxo-hexanoic acid, which is able to bear different fluorochromes and mimics the well known protecting group, the 4-oxo-pentanoic acid (levulinic acid) [2]. A treatment using a hydrazine-pyridinium acetate buffer at room temperature and at neutral pH leads to its removal after a few minutes. The triphosphate of the thymidine acylated with this analog has been synthetized [3].

During a previous study, we have demonstrated that 3'-O-acylated nucleoside triphosphate bearing fluorescent probe via a spacer were good substrates for DNA polymerases. Unfortunatly, these compounds were shown to be terminators of DNA synthesis only under particular conditions and according to the DNA polymerase used [4].

The incorporation of the new 3'-esterified triphosphate has been realized with the *Taq* DNA polymerase and the Klenow fragment exo⁻. The same results were obtained.

Incorporation catalyzed by *Taq* DNA polymerase



AACCC

Incubation for 3 min at 50°C

0,9 U/µL of enzyme,

[primer/template] = 80nM,

[ddGTP] = 1/10 [dTTP-3'-O-ester].

We have observed that two nucleotides were incorporated. This means that the 3'-ester is deprotected during the extension. Since the ester is stable in the reaction buffer, we can conclude that the DNA polymerase removes this 3'-ester too but only if the next correct nucleotide is present.

Complementary studies are now in progress.

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