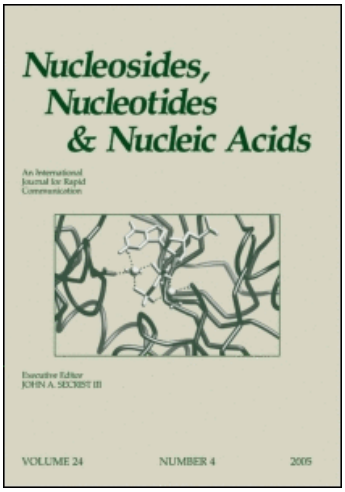

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STEP-BY-STEP CONTROL BY MALDI-TOF MS OF AN OLIGONUCLEOTIDE SYNTHESIS ON STANDARD CPG SOLID-SUPPORT

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ABSTRACT

MALDI-TOF mass spectrometry was used to monitor DNA solid-phase synthesis on long-chain alkylamine controlled-pore glass (LCAA-CPG) with a standard succinyl linker between the solid support and the growing oligonucleotide.

Solid-phase synthesis on long-chain alkylamine controlled-pore glass (LCAA-CPG) with a succinyl linker (1) between the CPG and the growing oligonucleotide is the method of choice for producing oligonucleotides according to the phosphoramidite approach (2). However, evaluation of the efficiency of the synthesis and of the integrity of the oligonucleotide is only available at the end of the synthesis after cleavage of the succinyl linker and removal of nucleobase and phosphate protecting groups. Direct control over the basic elongation process, evidence about side reactions or incompleteness is not directly possible.

We recently reported (3) that MALDI-TOF MS allowed direct read-out of the nucleotide sequence and backbone determination of regular solid-supported oligonucleotides bearing P_v internucleoside linkages. We would like to report here that this analytical method can be used to characterize still anchored oligonucleotides carrying non-nucleosidic residues as well as P_{III} internucleosidic linkages

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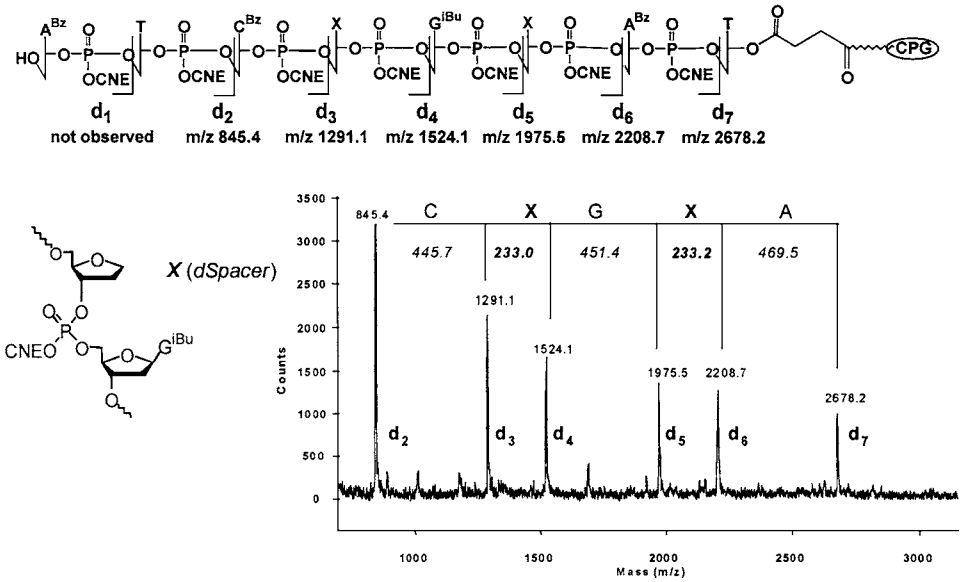

Figure 1.

(H-phosphonate and phosphite triester). As a consequence MALDI-TOF MS was used to follow the different chemical steps involved in the oligonucleotide elongation.

Analysis of a modified-base oligonucleotide

In 2,4,6 THAP as the matrix (4), a CPG-supported oligonucleotide bearing two dSpacer (5) modifications was directly analyzed by MALDI-TOF MS (Fig. 1). The resulting ladder of peaks (negative mode), that differ from another by the mass of nucleotide residues, revealed that laser irradiation induced fragmentation generated a set of d ions, representing the sequence of the oligonucleotide in the 5′ to the 3′ direction.

Analysis of P_{III} oligonucleotides

MALDI-TOF of solid-supported oligonucleotide H-phosphonate diesters showed similar d ion fragmentation pattern. Analysis of an oligonucleotide prepared with phosphoramidite chemistry showed a series of d ions bearing a phosphite triester linkage.

Monitoring elongation

During the synthesis of oligonucleotides on regular solid support by the phosphoramidite approach, MALDI-TOF analysis was directly performed on the solid

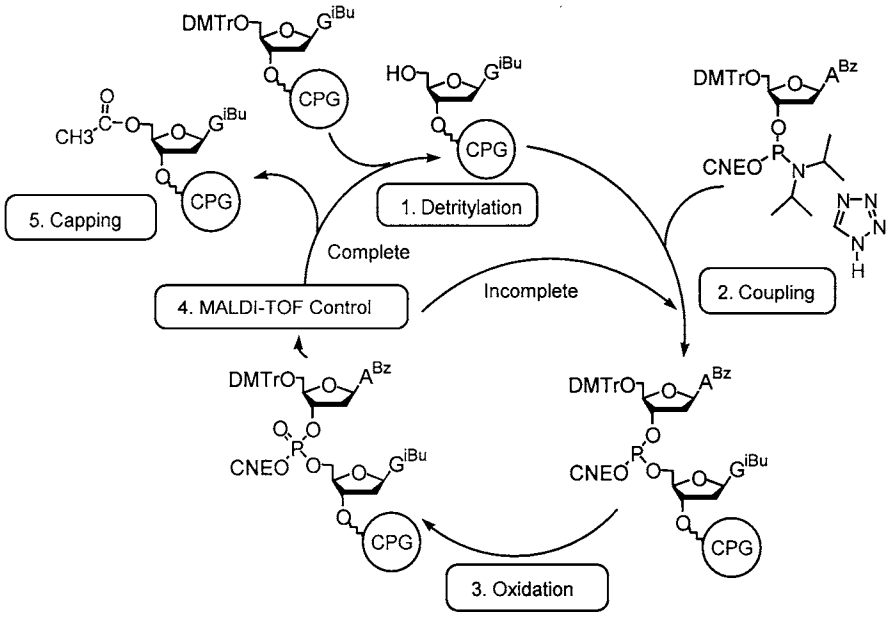

Figure 2. Optimization of a phosphoramidite coupling towards MALDI-TOF control.

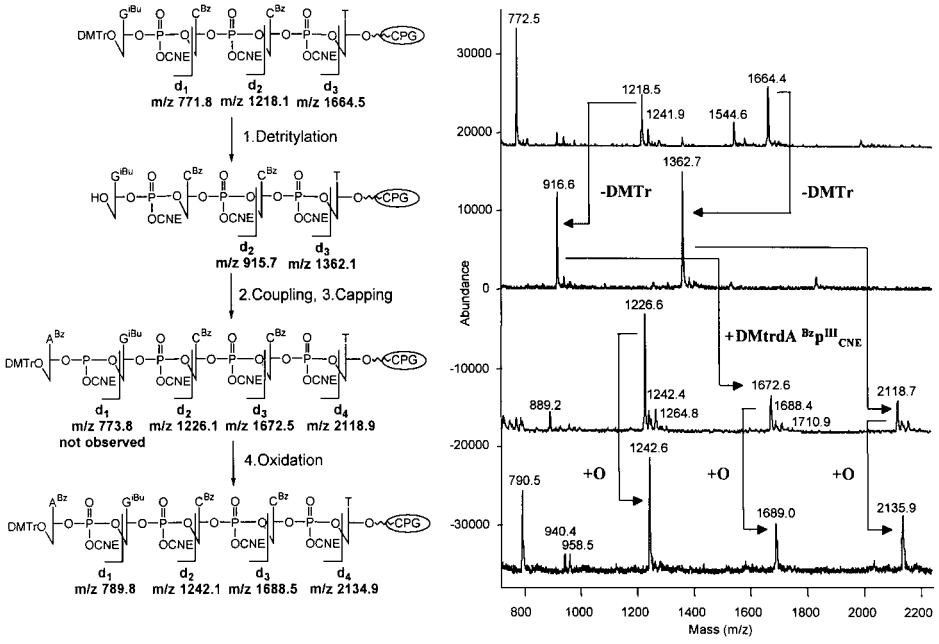

Figure 3.

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material (few beads) after each coupling-oxidatation steps. Mass of d_1 fragment carrying a 5′-DMTr protection unambiguously allowed direct reading of the incorporated nucleoside. Moreover, mass differences between d fragments gave information about the sequence integrity of the elongated oligonucleotide.

More interestingly, when this MALDI analysis was done between oxidation but before capping (Fig. 2), control of the coupling efficiency and optimization of this coupling was possible. Indeed, If coupling is inefficient, two series of d ions are observed, one corresponding to the starting material and the other one resulting from the reaction. The coupling step was repeated until disappearance of the signals of the starting material.

During the solid phase synthesis of $d^{5'}(AGCCT)^{3'}$ following the phosphoramidite approach, the different steps of the cycle of dA incorporation were monitored by MALDI-TOF MS (Fig. 3). Removal of DMTr resulted in the formation of a set of d ions loosing 302.4 Da when compared to the set of d ions of the starting oligonucleotide. The spectrum obtained after coupling of dA phosphoramidite showed ions containing a phosphite triester linkage. After oxidation, a new series of d ions that differ by 16 Da from the d series of the previous spectrum was observed.

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