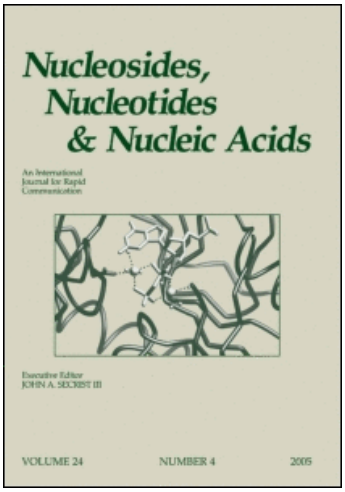

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Sequencing of Synthetic DNA Fragments Containing Various 5-Substituted Pyrimidines by Solid-Phase Chemical Degradation Using CCS Paper

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SEQUENCING OF SYNTHETIC DNA FRAGMENTS CONTAINING VARIOUS 5-SUBSTITUTED PYRIMIDINES BY SOLID-PHASE CHEMICAL DEGRADATION USING CCS PAPER

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Abstract. It is reported on solid-phase chemical degradation sequencing of more than 50 synthetic DNA fragments containing various 5-substituted pyrimidines including uracil (U), 5-fluorouracil (F^5U), 5-bromouracil (Br^5U), α and β anomers of 5-propyluracil (α-$prop^5U$ and β-$prop^5U$) and 5-methylcytosine (m^5C). Characteristic sequence patterns of oligonucleotides containing these base analogues are presented and their modification behaviour discussed.

Synthetic DNA fragments designed for biochemical or molecular biology investigations e.g. protein-DNA interaction [1-5] should be ultra pure. Despite of the large progress in the field of DNA chemistry, normal oligonucleotides are often modified due to the some times quite drastic conditions of the chemical synthesis. Furthermore, incorporated base analogues are additional hot spots at which side reactions can take place during synthesis and deblocking. It is, therefore, necessary to check each fragment by sequencing. Solid-phase chemical degradation using CCS anion-exchange paper is a powerful new sequencing technique to process many different DNA fragments [6-8] and to identify various 5-substituted pyrimidines e.g. U, F^5U, Br^5U, α- and β-$prop^5U$ and m^5C which have been found to be important contact points for restriction and modification enzymes [2-3].

The substituted uracil bases can be distinguished from each other or from thymine owing to different reactivity to oxidation with potassium permanganate (T>purine modification). Under identical conditions, F^5U is most strongly modified by $KMnO_4$ [9] followed by Br^5U > T, β-$prop^5U$ >

Part VI of a series entitled; Solid-phase methods for sequencing nucleic acids. For part V see [8].

α-prop^{5}U > U. On the other hand, alkaline conditions e.g. 10% piperidine at 90°C are likely to cause ring openings to a varying extent at the 5,6-double bond of Br^{5}U, F^{5}U and U follwoed by N-glycosidic bond cleavage in the respective nucleosides. As a result, the DNA chain is disrupted at the point of incorporation of these base analogues in the process of the piperidine reaction. Thus, additional bands can be identified in the G, A+G and C reactions at these positions. Br^{5}U and F^{5}U are most strongly attacked and, therefore, exibit the most intensive bands followed by U. In contrast, T and prop^{5}U are not attacked by piperidine or by more drastic alkaline conditions e.g. 1.2 M NaOH at 90°C.

Br^{5}U and U also react with hydroxylamine and show an additional band in the C-reaction. Qwing to the complex modification behaviour of Br^{5}U, F^{5}U and U, the observed final bands in the T>purines and C lanes are the result of different reactions. m^{5}C does not react with hydroxylamine at pH 6 and shows a characteristic gap in the sequence pattern [6,9].

The above results are also very useful for identifying certain base modifications of the synthetic fragment caused by the chemical synthesis. The heterocyclic bases thymine, adenine and guanine at the 3'-terminus of the chain were found to be more often modified than others, since they were most strongly exposed to the drastic conditions of the chemical synthesis (functionalisation and detritylation reactions).

It remains to be seen, if this method can be extended for identifying other C^5-, O^4- or C^6-substituted uracil, N^4- or C^5-substituted cytosine and various important purine bases, and combined with existing techniques for direct sequencing genomic DNA [10].

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