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Solid-phase methods for sequencing nucleic acids VIII. CCS paper-supported degradation of oligodeoxyribonucleotides containing 2'-deoxytubercidin

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Synthetic oligonucleotides with incorporated minor bases (modified pyrimidines and/or purines) are increasingly used for investigating various problems in molecular biology e.g. protein-DNA interactions[1-6]. Since modified nucleosides may represent additional hot spots at which side reactions can take place during chemical synthesis and deblocking, such synthetic fragments have to be strictly purified and characterized by a variety of analytical procedures.

Very recently, we have shown that solid-phase chemical degradation sequencing on CCS paper can be used to confirm the primary structure of synthetic DNA fragments containing various 5-substituted pyrimidines e.g. 5-methylcytosine, uracil, 5-fluoro-, 5-bromo-, and 5-propyluracil[7-8]. Due to different chemical behaviour during the degradation reactions, the above minor bases could be discriminated from each other and from thymine and cytosine by their individual band pattern. We now wish to report that chemical degradation sequencing can also be used to detect modified purines in synthetic oligonucleotides, and describe the characteristic band patterns for 2'-deoxytubercidin (7-deaza-2'-deoxyadenosine, c^7A_d).

Oligonucleotides d(GTAGc^7AATTCTAC) 1, d(GTAGAc^7ATTCTAC) 2, and d(GTAGAATTCTc^7AC) 3 have recently been synthesized[6]. Solid-phase chemical degradation on CCS paper was carried out according to Ref. 8 with two controls (lanes 1 and 2, fig. 1) and using two additional chemical modification reactions, A>G[9] and A>C[10] (lanes 3 and 4, fig. 1).

As shown in the figure, the correct primary sequences of the oligomers 1-3 were confirmed. 7-Deaza-2'-deoxyadenosine exhibits different properties with respect to adenine for most of the modification reactions and can, therefore, be easily identified by its specific band pattern. Compound c^7A_d does not react either with 10% piperidine (lanes 2) or with 1.2 M sodium hydroxide (lanes 4) at 90°C indicating stability of the 5-membered ring against alkaline conditions. By contrast, adenine is significantly degraded by NaOH under the above conditions, resulting in strong bands (lane 4). Compounds A_d and c^7A_d are equally weakly methylated by dimethyl sulphate at pH 3.5 as indicated by faint signals in the G lanes. Under these circumstances, methylation occurs presumably at the pyrimidine ring. Ethylation of adenine bases at the N-7 atom with diethylpyrocarbonate (DEPC) at pH 5.5[9] represents the main modification reaction (strong signals in lanes 3). By contrast, ethylation of 7-deazaadenine occurs only as a side reaction in the pyrimidine ring, resulting in weak bands. The highly stable N-glycolidic bond of c^7A_d is only slowly degraded by formic acid, giving rise to weak bands in the A+G lane. Adenine is more readily attacked by the acid, giving stronger bands. The bases show different susceptibilities to modification with potassium permanganate. Deoxyadenosine does not react with $KMnO_4$, but c^7A_d is slightly oxidized and exhibits a detectable band in the T>Creaction. Presumably, the oxidation of 7-deazaadenine occurs via a hydroxylation reaction at the C-5/C-6 double bond of the pyrrole ring.

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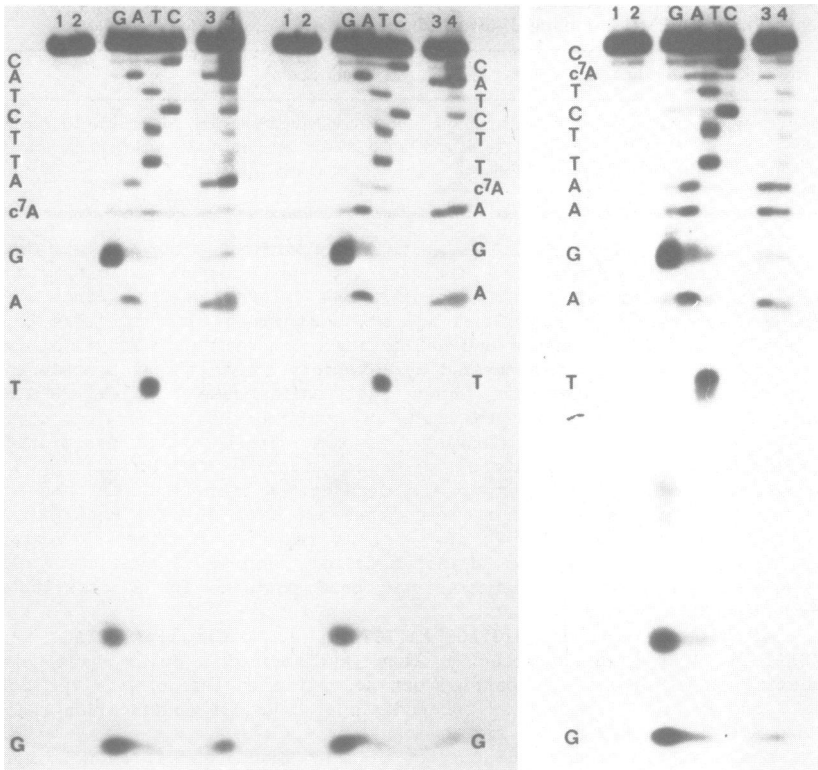

Fig. 1 CCS paper-supported degradation of three oligonucleotides containing 7-deazaadenine at various positions of the chain. Lanes 1: purified oligomers 1-3 as controls; lanes 2: the same oligomers after piperidine treatment (1M, 30 min at 90°C) and lyophilization; lanes G, A+G, T>C and C: solid-phase modification reactions on CCS paper with DMS, HCOOH, $KMnO_4$, and NH_2OH, respectively, according to Rosenthal et al.[8], followed by piperidine treatment and lyophilization; lanes 3: solid-phase A>G modification on CCS paper with 10% (v/v) DEPC in ethanol at 90°C for 10 min adapted from Kraev[9], followed by piperidine reaction at lyophilization; lanes 4: A>C modification in solution according to Maxam and Gilbert[10], follwoed by piperidine reaction and lyophilization.

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