A CONFORMATION-SPECIFIC CHEMICAL MODIFICATION OF NUCLEIC ACIDS

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Small molecules containing an adenine ring react under mild conditions with α -acetylenic esters. Chlorotetrolic (4-chlorobutynoic) acid ester yields two isomeric products $\underline{1}$ and $\underline{2}$ $[1-\overline{3}]$.

However, in the case of nucleic acids, the nature of the isomer formed depends mainly on steric factors. Thus, treatment of poly(A) with methyl chlorotetrolate at pH 7 leads first to derivatives of type $\underline{1}$ instead of the normally expected $\underline{2}$ isomers. This chemical modification is accompanied by a gradual destacking of the bases which makes possible the formation of derivatives $\underline{2}$ in the later stages of the reaction when the conformation of the polynucleotide chain changed sufficiently.

In the case of $tRNA^{Phe}$ the reaction involves the anticodon loop and the 3'-end. In the anticodon region which has an ordered conformation with nearly normal base stacking [4], adenine bases are converted to derivatives of structure 1, while in the 3'-end in which the bases are only slightly stacked [4], isomers 2 are formed. Derivatives 1 and 2 have very different physical properties and can be easily identified e.g. by HPLC following hydrolysis of modified nucleic acids.

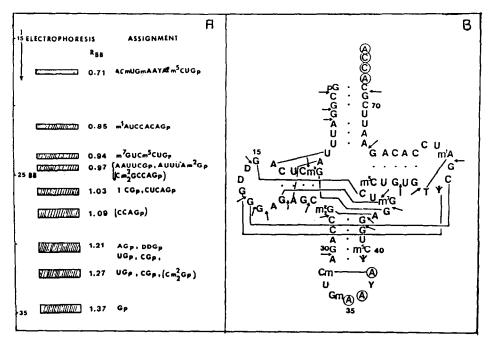


Fig. 1. A Scheme of electrophoretic separation of T_1 fragments of modified tRNA. $\overline{20}$ % polyacrylamide - 7 M urea; BB; spot of bromophenol blue, B. Cloverleaf diagram of yeast tRNA^{Phe} showing the sites of modification (circles) and of hydrolytic cleavage with ribonuclease T_1 (arrows).

Thus, the characteristics of the reaction of chlorotetrolic acid esters with adenine rings inserted in a polymer depend on the conformation of the latter, presumably because of the sterical requirements of the linear and rigid reagent.

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