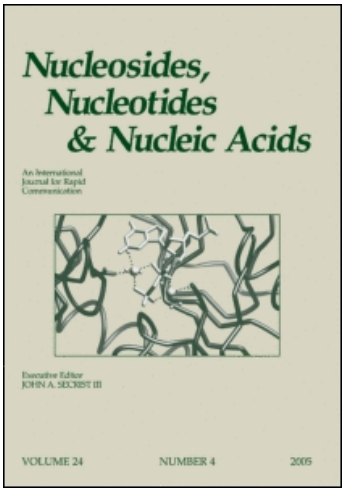


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# Ethidium Oligodeoxynucleotide Derivatives. Stability Against Cellular Nuclease Hydrolysis and Photochemical Modification of Cellular Proteins in the Living Cells

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# ETHIDIUM OLIGODEOXYNUCLEOTIDE DERIVATIVES. STABILITY AGAINST CELLULAR NUCLEASE HYDROLYSIS AND PHOTOCHEMICAL MODIFICATION OF CELLULAR PROTEINS IN THE LIVING CELLS

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ABSTRACT. Oligonucleotide derivatives with ethidium residues attached to 3'- or 5'-end demonstrated enhanced stability against cellular nuclease hydrolysis when being incub ated with living cells. Irradiation of the cells with UV-light (>300 nm) in the presence of 5'-ethidium decathymidylate derivative at 4°C resulted in the specific modification of protein(s).



Oligonucleotide derivatives containing reactive groups are used as effective reagents for sequence specific modification of nucleic acids [1]. Among of the most promising are the derivatives containing photoreactive group stabilizing complementary complexes such as ethidium [2], phenazinium [3] and acridinium dyes [4]. To study a stability of ethidium derivatives (as well as alkylating 4-N-2-chloroethyl,N-methylbenzylphosphamido derivatives) against cellular nuclease hydrolisis the above oligonucleotide derivatives were incubated with Krebs-2 cells (Table). It is seen that 5'-derivatives were more stable than unsubstituted decathymidylate ( R = R'=p). They were also more stable than 3'-derivatives indicating the presence of strong 5'-nuclease activity for Krebs-2 cells.

The experiments on alkylation of the intracellular biopolymers (data not presented) with decathymidylate derivatives (I) and (II) containing alkylating group demonstrated that these derivatives penetrated the cell membranes. Among the targets modified with the above derivatives were RNA and DNA (protein fraction was not checked).

The most interesting results were achieved in the preliminary experiments on photomodification of the cellular biopolymers with decathymidylate derivative (Ia, R= pNHROH, R'= pNHBtd-2). The mixture of ascite tumor Krebs-2 cells and the above derivative was irradiated with ultraviolet light (>330 nm) at 4 and at 20°C. The results are presented in FIG. Only weak bands were detected in the autoradiogram of PAG protein electrophoresis in the experiments at 20°C (lane 3). A strong and several weaker bands of modified proteins were detected in the experiment at 4 C (lane 4). The nature of the protein(s) modified is being investigated now.

Table

Stability of the decathymidylate derivatives incubated in the presence of the ascite tumor Krebs-2 cells in culture medium.

| Derivative of (R)TTTTTTTTTT(R') 5 uM, 5'-32-P labeled) | | Percentage of the intact decanucleotide derivative (accuracy 10%) 1h | 24h |
|---|---|---|---|
| (I) R=pNHRCl, R'=pNHEtd-2 | Cells | 93 | 84 |
| | Medium | 85 | 90 |
| (II) R=pNHRCl, R'=p | Cells | 94 | 82 |
| | Medium | 88 | 79 |
| (III) R=p, R'=p | Cells | 21 | Traces |
| (IV) R=p, R'=pNHEtd-2 | Cells | 58 | 19 |
| | Medium | 39 | 6 |
| (V) R=pNHEtd-2, R'=OH | Cells | 95 | 95 |

* Cells (5 million cells/ml) were incubated at 37°C with decathymidylate derivatives in DMEM (FLOW Laboratories). Medium was removed, the cells washed and lysated. The cell lysate and medium were analysed using the PAG electrophoresis. The percentage of intact derivative was estimated by comparison of the radioactivities of the respective spots.

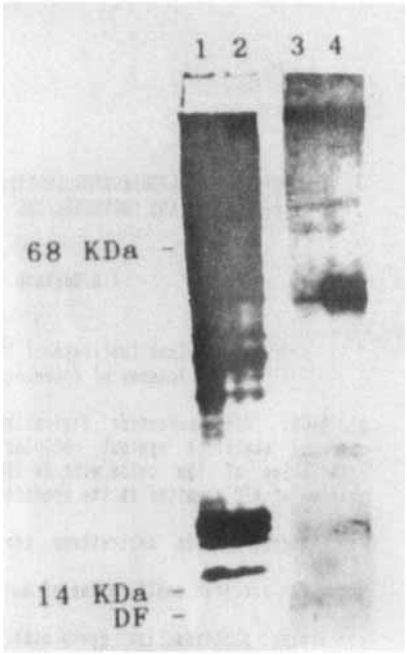


FIGURE. SDS-PAG gel electrophoresis of isolated cellular proteins fractions of Ascite tumor Krebs-2 cells on 12% gel stained with Coomassie Brilliant Blue G-250. The cells were irradiated in the presence of oligonucleotide derivative (Ia, R=pNHROH, R'=pNHEtd-2) at 20°C (lane 1) and at 4°C (lane 2). Lanes 3 and 4 are the autoradiograms of lanes 1 and 2 respectively. DF-dye front.

The results presented demonstrate that oligonucleotide derivatives with covalently attached ethidium residues can be considered as potential instrument in the specific photomodification of native cellular biopolymers.

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