ANTISENSE OLIGONUCLEOTIDES MODIFIED AT 5'-END BY FATTY RADICALS EFFECTIVELY INHIBIT REPRODUCTION OF INFLUENZA VIRUS

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Received October 20, 1989
Accepted October 23, 1989

To enhance penetration of oligonucleotides ("oligos") into cells it was suggested that they should be chemically modified by fatty radicals at 5'-end. Two modified by undecanol at 5'-end phosphate group oligos, namely, oligo complementary to the protein binding sites, located at the influenza virus polymerases encoding RNA, and oligo complementary to the polyadenylation signal of the polymerase 3 encoding RNA were synthesized using DNA-synthetisator. These modified oligos effectively suppressed the influenza A/PR8/34 virus reproduction and inhibited the synthesis of virus-specific proteins in MDCK cells. The non-modified antisense oligos and modified nonsense oligos did not affect the virus development under the same conditions.

The antisense oligonucleotides ("oligos") are regarded as one of the most perspective tools for the regulation of transcription and translation processes[1]. However, the application of oligos is considerably limited by the low efficiency of their penetration into intact cells. It has been demonstrated recently[2-4] that water-soluble proteins can be transformed into a state capable of translocating across biomembranes and penetrating into cells via their chemical modification by hydrophobic anchor groups – fatty acid residues. In this work we suggest introducing the fatty radicals at the 5'-end of oligos for increasing their effect on the cells.

We synthesized two oligos at a DNA-synthetisator ("Applied Biosystems", Model 380 B-02) using 2-cyanoethylphosphoramidite building blocks[5], namely, TTGACGAAATT (*I*), which presumably is complementary to the protein binding sites, located at influenza virus polymerases encoding RNA, and ATTTTATTGTA (*II*), which is complementary to the polyadenylation signal of polymerase 3 encoding RNA[6].

The undecyl radicals were introduced at the 5'-ends of the growing chains during the synthesis of these oligos by the addition of 1-undecyl-2-cyanoethyldiisopropyl-

amidophosphine, which was synthesized by a modified method with the application of 2-cyanoethyl-N,N,N′,N′-tetraisopropylphosphorodiamidite[7].

We investigated the effect to thus modified antisense oligos on the reproduction of influenza A/PR8/34 virus in permissive MDCK cells using the plaque assay method[7]. As can be seen in Fig. 1 the modified oligo *I* considerably suppressed the development of the virus infection (the number and the size of the plaques), whereas non-modified oligo *I* does not show such effect at the same concentrations. The analogous result (data not shown) was observed for oligo *II*: the modified oligo unlike the non-modified one inhibited the virus reproduction. It should be stressed that modified nonsense oligos did not affect the virus reproduction, i.e. the observed effect results from the specific interaction of antisense oligos with the corresponding sites of RNA.

By an example of high multiplicity of infected MDCK cells (10 plaque forming units/cell) it was demonstrated that oligos (both *I* and *II*) modified by undecyl radicals effectively inhibit the synthesis of virus-specific proteins. The non-modified oligos do not affect this process under the same conditions. The data obtained permit us to suppose that 5′-end modified by fatty radicals antisense oligos can become a new class of antivirals and agents for the regulation of intracellular processes.

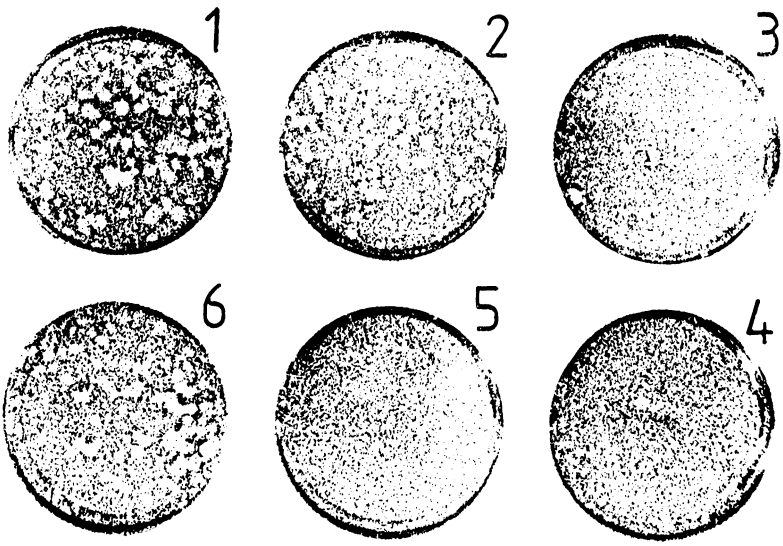

FIG. 1

Plaque formation in the monolayer of MDCK cells infected by influenza A/PR8/34 virus[8] in the media containing: 1 no oligos; 2 10 μmol l^{-1}; 3 50 μmol l^{-1}; 4 100 μmol l^{-1}; 5 200 μmol l^{-1} of modified oligo *I*; 6 200 μmol l^{-1} of non-modified oligo *I*

This work was supported by the grant "Cell-free protein synthesis" of the U.S.S.R. State Committee of Science and Technique. We thank V. S. Pheoktistov for the qualified technical assistance.

REFERENCES

1. Toulmé J.-J., Hélène C.: Gene *72*, 51 (1988).
2. Kabanov A. V., Nametkin S. N., Levashov A. V., Martinek K.: Biol. Membrany (Russ.) *2*, 985 (1985).
3. Kabanov A. V., Levashov A. V., Alakhov V. Yu., Kravtsova T. N., Martinek K.: Collect. Czech. Chem. Commun. *54*, 835 (1989).
4. Kabanov A. V., Ovcharenko A. V., Melik-Nubarov N. S., Bannikov A. I., Alakhov V. Yu., Kiselev V. I., Sveshnikov P. G., Kiselev O. I., Levashov A. V., Severin E. S.: FEBS Lett. *250*, 238 (1989).
5. Gait M. G. (Ed.): *Oligonucleotide Synthesis: A Practical Approach.* IRL Press, London 1984.
6. Kaptain J. S., Nayak D. P.: J. Virol. *42*, 55 (1982).
7. Nielsen J., Taagaard M., Marugg J. E., van Boon J. H., Dahl O.: Nucleic Acids Res. *14*, 7391 (1986).
8. Zhirnov O. P., Ovcharenko A. V., Bukrinskaya A. G.: Arch. Virol. *71*, 177 (1982).