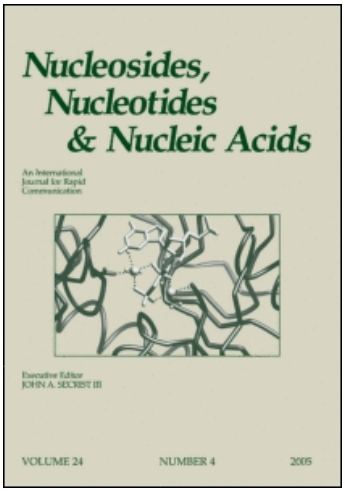

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E. Uhlmann[a]; A. Peyman[a]
[a] Hoechst Marion Roussel, Chemical Research, Frankfurt a. M., Germany

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THE CONCEPT OF 'MINIMALLY MODIFIED' ANTISENSE OLIGONUCLEOTIDES FOR SPECIFIC INHIBITION OF GENE EXPRESSION

E. Uhlmann* and A. Peyman
Hoechst Marion Roussel, Chemical Research G 838, D-65926 Frankfurt a. M., Germany

ABSTRACT: The design and use of minimally modified oligonucleotides for specific inhibition of gene expression is discussed. The "minimal" protection strategy is a combination of the end-capping technique and the protection of internal pyrimidine positions which are the major sites of endonuclease degradation. By reducing the number of phosphorothioate modifications needed to make the oligonucleotide resistant to nuclease degradation, non-sequence-specific effects, which are frequently observed with uniformly phosphorothioate-modified oligonucleotides, can be reduced.

One of the major problems encountered using uniformly phosphorothioate (PS) modified oligonucleotides (ODN) is their propensity for non-antisense effects, such as the inhibition of DNA polymerases, RNase H, protein kinases, as well as binding to growth factors and to cell surface receptors [1,2]. Therefore, it is desirable to reduce the number of PS linkages within ODN to a minimum which is necessary to stabilize against nucleotlytic degradation. Although the major degrading activity in serum is brought about by a 3'-exonuclease, terminal modification, such as 3'- or 3'5'-end-capping of ODN turned out not to be an adequate protection strategy, as the end-capped ODN are still subject to degradation by endonucleases [3].

The "minimal protection" strategy [4] involves the following design considerations: (i) The ODN is end-capped by two to five PS residues at the 3'-end to render the ODN stable against 3'-exonucleases. (ii) The ODN is end-capped by one or two PS residues at the 5'-end to render the ODN stable against potential 5'-exonuclease cleavage.

(iii) Additional PS linkages are placed at internal pyrimidine nucleotides which are the major sites of degradation by endonucleases. In the case of two or more adjacent pyrimidines, this internal protection becomes especially important. (iv) More than about five PS residues in a row should be avoided, in order not to run into the side-effects observed for all-phosphorothioates. We have found that ODN, in which about 40 to 60% of the internucleotide linkages were PS-modified according to this strategy, showed similar stability in serum and within cell extracts as uniformly PS-modified ODN.

5'- N * N * N Pu Py * Pu Py * Py * Py Pu Py * N * N * N-3'

General structure of a minimally PS-modified oligonucleotide
(is phosphorothioate;* Py *is a pyrimidine and* Pu *is purine nucleotide)*

Studies on the mechanism of stabilization of these minimally PS-modified ODN suggest that their nuclease stability is not only caused by direct prevention of nuclease attack at the phosphate centre, but is additionally supported by interference of the nucleases with the PS groups of ODN resulting in decreased degradation [5]. Interestingly, the stability of antisense ODN correlates with their biological activity [6]. Replacement of internal pyrimidine nucleotides by 5-(hexynyl) analogs was found to result in improved binding affinity, increased stability towards nucleases and enhanced biological activity [7]. The minimal protection scheme has also been successfully applied to antisense ODN directed against different targets, such as c-*cbl*, c-*src* [8] , c-*fos* [9], c-*myc* [10], and TNFRI [11], resulting in highly specific inhibition of gene expression in cell-based assay systems.

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