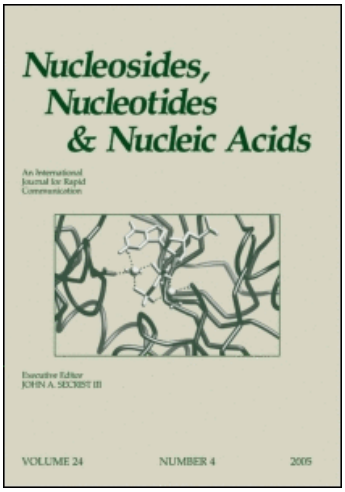

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2',5'-PHOSPHODIESTERASE ACTIVITY DEPENDS UPON THE PRESENCE OF A 3'-HYDROXYL MOIETY IN THE PENULTIMATE POSITION OF THE OLIGONUCLEOTIDE SUBSTRATE

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Abstract. 3'-Deoxyadenosine-substituted analogs of 2-5A core 5'-monophosphate were examined for their degradation by the 2'-phosphodiesterase of mouse cells, leading to the conclusion that the 2',5'-phosphodiesterase requires the presence of 3'-hydroxyl moiety in the penultimate nucleotide.

INTRODUCTION :

2-5A(ppp5'A2'p5'A2'p5'A) an established mediator of some antiviral activities of interferon can be inactivated by cleavage of the phosphodiester bonds by a 2',5-phosphodiesterase activity that appears to be present in most cells which have been examined. This enzymatic activity produces 5'-AMP from internal and 2'-terminal nucleotides and 5'-ATP from the 5'-terminus of the oligonucleotide chain, and requires Mg^{+2} for activity. Relatively little has been reported relating the structure of the potential oligonucleotide substrate and its ability to be degraded by the 2',5'-phosphodiesterase activity. Herein we present the first evidence that degradation by the 2',5'-phosphodiesterase activity requires a 3'-hydroxyl group in the penultimate nucleotide of the 2',5'-oligomer.

RESULTS :

The degradation of various 3'-deoxyadenosine-substituted analogs of 2-5A 5'-monophosphate was studied under conditions of protein synthesis since such conditions are used to evaluate the biological activity of such analogs. It was clear that, under such conditions, followed order of stability (most stable to least stable) prevailed: p5'(3'dA)2'-p5'(3'dA)2'p5'(3'dA) ($t_{1/2}$ >>120 min) ∿p5'A2'p5'(3'dA)2'p5'A ($t_{1/2}$ >>120 min) >> p5'A2'p5'A2'p5'(3'dA) ($t_{1/2}$ = 140 min) > p5'(3'dA)2'p5'A2'p5'(3'dA) ∿p5'(3'dA)2'p5'A2'p5'A ($t_{1/2}$ = 90 min) ≥p5'A2'p5'A2'p5'A ($t_{1/2}$ = 70 min).

CONCLUSIONS :

The most direct conclusion from the data is that the simple presence of a 3'-deoxyadenosine residue in a 2',5'-oligoadenylate does not confer maximum resistance to degradation. It is the 3'-hydroxyl moiety of the second (from the 2'-terminal) or penultimate nucleotide unit of p5'A2'p5'A2'p5'A that is required for cleavage by the 2',5'-phosphodiesterase activity of mouse L cell extracts. At present, we cannot discriminate whether this hydroxyl group is needed for phosphodiesterase enzyme recognition or because a cyclic phosphate intermediate is involved in the mechanism of phosphodiesterase action. There may be at least three reasons for the necessity of a 3'-hydroxyl group in the penultimate nucleotide: (a) it may be required for phosphodiestease enzyme recognition; (b) a cyclic phosphate intermediate could be involved in the mechanism of phosphodiesterase action; (c) the 3'-hydroxyl functionalizing of th epenultimate nucleotide may alter the reaction course by significantly altering the conformation of the oligonculeotide substrate as in a reaction transition state.