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3'-FLUORO-3'-DEOXY ANALOGS OF 2-5A 5'-MONOPHOSPHATE: BINDING TO 2-5A-DEPENDENT ENDORIBONUCLEASE AND SUSCEPTIBILITY TO (2'-5')PHOSPHODIESTERASE DEGRADATION

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Analogs of 2-5A trimer 5'-monophosphate (2'-5')pA$_3$,p5'A2'p5'A2'p5'A containing 9-(3-fluoro-3-deoxy-c-D-xylofuranosyl)adenine (A^F) or 3'-fluoro-3'-deoxyadenosine (A_F) at different positions of the chain have been synthesized. All of them were compared with (2'-5')pA$_3$ and (2'-5')pA$_2$(3'dA) by (i) their ability to bind to 2-5A-dependent endoribonuclease(RNase L) of mouse L cells and of rabbit reticulocyte lysates and (ii) their susceptibility to the degradation by the (2'-5')phosphodiesterase activity. The results of this study suggest that the oligonucleotide conformation is important for its biochemical properties. © 1990 Academic Press, Inc.

Studies on relative importance of each of the ribose 3'-hydroxyl groups of 2-5A trimer 5'-monophosphate [(2'-5')pA$_3$, p5'A2'p5'A2'p5'A] in determining the binding to 2-5A-dependent endoribonuclease (RNase L), using three analogs in which one of the nucleotide fragment was sequentially replaced by 3'-deoxyadenosine (3'dA, cordycepin), showed that the 3'-hydroxyl group of the penultimate nucleotide residue of (2'-5')pA$_3$ makes major contribution to the binding of (2'-5')pA$_3$ to RNase L (1). Moreover, the same three analogs were tested for their susceptibility to the (2'-5')phosphodiesterase activity (2). It was found, that replacement of 3'-hydroxyl group of the penultimate nucleotide fragment of the parent oligoadenylate, (2'-5')pA$_3$, by hydrogen gave an analog, (2'-5')pA(3'dA)A, which was completely resistant to degradation. Two other analogs were not significantly more resistant to the (2'-5')phosphodiesterase action than the parent (2'-5')pA$_3$ was (2). Based on these results, Torrence et al. have hypothesized

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(2) that the 3'-hydroxyl group of the penultimate nucleotide may be involved either in the substrate-enzyme recognition or in the formation of an intermediate 2',3'-cyclic phosphate. This is in agreement with the observation that a change in the 3'-hydroxyl group configuration from _ribo_ to _xylo_ in the penultimate nucleotide of (2'-5')pA_3 resulted in the analog which was resistant to the (2'-5')phosphodiesterase activity (3). In addition, it was also suggested (2) that the 3'-hydroxyl group under consideration may play an important role in the formation of the proper oligonucleotide conformation in the productive enzyme-substrate complex. To the best of our knowledge, there was no evidence that the conformational factor may play such an important role.

We wished to study the role of the conformation of (2'-5') pA_3 in the binding to 2-5A-dependent RNase L and in the degradation by the (2'-5')phosphodiesterase activity using analogs containing 9-(3-fluoro-3-deoxy-c-D-xylofuranosyl) adenine (A^F) or 3'-fluoro-3'-deoxyadenosine (A_F) at different positions of the trimer 5'-monophosphate.

Materials and Methods

The preparation of mouse L_{929} cell extracts and rabbit reticulocyte lysates was perfomed according to Torrence and Friedman (4). The probe used for radiobinding assays, (2'-5')$p_3A_4$3'[^{32}P]p5'C3'p, was synthesized and purified by HPLC as described by Knight et al. (5). Radiobinding assays were performed as described by Knight et al. (5); the data are summarized in Table.

Degradation of the oligonucleotides was assayed under protein synthesis conditions at 30°C for 30 to 180 min as described by Torrence et al. (2). The concentration of added oligonucleotides was $1.2 \pm 0.1 \times 10^{-4}$ M. Aliquots were withdrawn and reactions were terminated by heating at 100°C for 5 min, samples were cooled to 20°C and centrifuged for 5 min at 10,000 x g and the supernatants were analyzed by HPLC (Bruker Liquid Chromatography System, FRG) on a reverse-phase column (Zorbax ODS C18, 15 x 0.46 cm).

Synthesis of 9-(3-fluoro-3-deoxy-c-D-xylofuranosyl)adenine and 3'-fluoro-3'-deoxyadenosine will be published elsewhere. Synthesis of the oligonucleotide 5'-monophosphates was performed according to previously published methodology (6-8) and will be described in a full paper. The purity of all synthesized oligomers was checked by HPLC, further confirmed by ^{1}H NMR spectroscopy (Bruker WM-360, FRG) and by degradation to the corresponding "core" trimers under the action of alkaline phosphatase.

Results

The binding affinity of all synthesized oligomers to 2-5A-dependent endoribonuclease (RNase L) of mouse L cells and rabbit reticulocyte lysates was studied according to the methodology by Knight et al. (5). This binding assay is based

Table 1. Interaction of the fluorodeoxy analogs of 2-5A with RNase L as determined by radiobinding assay

(2'-5')oligo-adenylates	HPLC retention time (min)	IC_{50}, M 10^{-8}*			
		mouse L cells	relative ability	rabbit reticulocyte	relative ability
pA_3	11,05	0,7	1,0	2,5	1,0
pA_2(3'dA)	12,58	0,8	0,9	20	0,125
$p(A^F)A_2$	10,46	2,0	0,35	5,5	0,45
$pA(A^F)A$	13,10	1,5	0,47	50	0,05
$pA_2(A^F)$	13,12	2,0	0,35	50	0,05
$p(A_F)A_2$	15,26	2,0	0,35	9	0,28
$pA(A_F)A$	14,18	0,6	1,2	10	0,25
$pA_2(A_F)$	14,49	0,2	3,5	13	0,2

* The values presented are mean values of 3-7 experiments.

on the displacement of the radiolabeled probe, (2'-5')$p_3A_4$3'[^{32}P]p5'C3'p, from an endoribonuclease-nitrocellulose complex (5). The results are expressed in terms of the concentrations necessary to prevent binding of 50% of the radiolabeled probe to the RNase L, *viz.*, IC_{50} values, and are summarized in Table 1. Remarkably, the IC_{50} values for (2'-5')pA_3 and (2'-5')pA_2(3'dA) are 7-8-fold greater than those reported by Torrence et al. (1). The discrepancies between our results and previously published ones may reflect some differences in the preparation of mouse L cells extracts. At the same time, a comparative analysis of the changes of IC_{50} values for the compounds studied in the present paper with those of Torrence et al. (1) seems to be correct. Furthermore, in the case of RNase L of rabbit reticulocyte lysates, the IC_{50} value for (2'-5')pA_3 is in agreement with an earlier study (9).

The degradation of the trimers by the (2'-5')phosphodiesterase activity in cytoplasmic extracts of mouse L cells was monitored by HPLC as illustrated by Fig.1 where the decrease in trimer 5'-monophosphate is presented. The degradation rates of (2'-5')pA_3 and (2'-5')pA_2(3'dA) are in

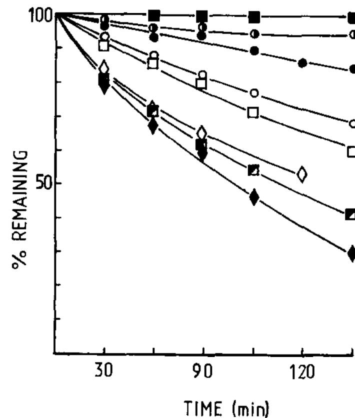

Figure 1. Degradation of the fluorodeoxy analogs of 2-5A by the (2'-5')phosphodiesterase activity of mouse L cells. The relative concentrations of oligonucleotides were determined from corresponding peak area integrations. The legend is as follows: ◆, pA_3 ; ◩, pA_2(3'dA) ; ○, $p(A^F)A_2$; ◑, $pA(A^F)A$; ●, $pA_2(A^F)$; □, $p(A_F)A_2$; ◇, $pA(A_F)A$; ■, $pA_2(A_F)$.

reasonable agreement with the data (2) for the same trimers. It should also be noted that we have not observed 5'-dephosphorylation of the trimers studied under the assay conditions.

Discussion

From the structural point of view, the two sets of the A^F or A_F sequentially substituted analogs of 2-5A trimer 5'-monophosphate are closely related to the corresponding 3'-deoxyadenosine analogs studied by Torrence et al. (1,2). Indeed, Van-der-Waals radii of fluorine and hydrogen atoms are 1.35 and 1.20 A, respectively, and the steric hindrances created by the former atom are therefore practically inappreciable. Consequently, it might be expected that the biological properties of each pair of *ribo* and *xylo* 3'-fluoro-3'-deoxy analogs should correlate with those of the related 3'-deoxy analog. However, we have found essential differences in the binding of these analogs to 2-5A-dependent RNase L and in the susceptibility to the (2'-5')phosphodiesterase activity.

First of all, it should be noted that the ability of 3'-fluoro-3'-deoxy analogs to bind to RNase L from both sources

depends on the configuration of fluorine atom that , in turn, leads to the alteration of stereochemistry of fluorodeoxy nucleoside and consequently the entire oligonucleotide chain.[1] Thus, (2'-5')pA_2(A^F) bound to RNase L one order of magnitude less tightly than (2'-5')pA_2(A_F). Moreover, there were essential differences in the binding ability of these *xylo* and *ribo* fluorodeoxy analogs and the parent substrate, (2'-5')pA_3, and its 3'-deoxy analog, (2'-5')pA_2(3'dA). The latter two oligomers were found to be almost equally effective in the displacement of the radiolabeled probe (Table), which is in reasonable agreement with an earlier study (1). Furthermore, when 3'-hydroxyl group of the central adenosine fragment was replaced by fluorine atom in the *ribo* configuration, the resulting analog, (2'-5')pA(A_F)A, bound to RNase L with equal affinity as (2'-5')pA_3. However, the replacement of the same hydroxyl group by fluorine atom in the *xylo* configuration resulted in analog, (2'-5')pA(A^F)A, which bound to the enzyme only half as effectively as the parent oligomer. These results give an extra evidence for the importance of the stereochemistry of trimers for the binding process. Unexpectedly, the relative ability of both analogs, (2'-5') pA(A_F)A and (2'-5')pA(A^F)A, to bind to RNase L (Table) differed significantly from that of (2'-5')pA(3'dA)A (1). Finally, the replacement of 3'-hydroxyl group of the 5'-terminal nucleotide residue of (2'-5')pA_3 by fluorine atom brought about the analogs, the binding ability of which was ca. three times lower than that of the parent trimer 5'-monophosphate independently of the fluorine configuration. In contrast, 3'-deoxyadenosine analog, (2'-5')p(3'dA)A_2, bound to RNase L two times more effectively than (2'-5')pA_3 (1).

There are no similarities between the relative ability of each isomeric pair of *ribo* and *xylo* fluorodeoxy analogs to bind to RNase L of mouse L cells and rabbit reticulocyte lysates, except for the trimers that bore a fluorodeoxy nucleoside at the 5'-terminus, *viz.*, (2'-5')p(A_F)A_2 and (2'-5')p(A^F)A_2. At the same time, the differences in the IC_{50}

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[1] Preliminary examination of stereochemistry of the isomeric pairs of fluorodeoxy analogs by ^{1}H NMR and CD have revealed the considerable conformational differences of *ribo* and *xylo* analogs.

values for (2'-5')pA_2(A_F), (2'-5')pA_2(A^F) and (2'-5')pA_2(3'dA) reflect mainly the differences in the stereochemistry of the trimers.

The rate of degradation of the trimers (2'-5')p(A^F)A_2, (2'-5')pA(A^F)A, (2'-5')pA_2(A^F) and (2'-5')p(A_F)A_2 by the (2'-5')phosphodiesterase activity reasonably correlates with that of the corresponding 3'-deoxy analogs (2). Unexpectedly, the substrate properties of (2'-5')pA(A_F)A and (2'-5')pA_2(A_F) were found not to correlate with that of the corresponding 3'-deoxy analogs (2). These findings suggest that the conformational factor may play an important role in hydrolysis process. Besides, the absence of 3'-hydroxyl group in the middle position of a trimer is not an only factor which *per se* induces resistance to the action of phophodiesterase. Finally, the replacement of 3'-hydroxyl group of the 2'-terminal nucleoside residue of (2'-5')pA_3 by fluorine atom in the *xylo* configuration or, to a greater extent, in the *ribo* configuration brought about the phosphodiesterase resistance. It is noteworthy that the inversion of 3'-hydroxyl group configuration of the 2'-terminal unit of a trimer to give *xylo* analog did not influence the rate of its degradation (3). On the other hand, the presence of 9-(c-D-arabinofuranosyl)adenine at the 2'-terminus of a trimer resulted in the analog which was completely resistant to (2'-5')phosphodiesterase activity (10).

Of particular interest is our observation that the analog (2'-5')pA_2(A_F) with a high binding affinity to RNase L of mouse L cells was found to be among the most stable substances towards phophodiesterase degradation.

Our further studies will be focused on the synthesis of 5'-triphosphates of the fluorodeoxy analogs and on the investigation of RNase L activation process.

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