Volume 212, number 2, 267–270 FEB 04463 February 1987

# Degradation by the 2′,5′-phosphodiesterase activity of mouse cells requires the presence of a *ribo* hydroxyl group in the penultimate position of the oligonucleotide substrate

Paul F. Torrence, David Alster, Sophie Huss+, Gilles Gosselin+ and Jean-Louis Imbach+

*Section on Biomedical Chemistry, Laboratory of Analytical Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, US National Institutes of Health, Bethesda, MD 20892, USA* and +*Laboratoire de Chimie Bio-Organique, UA no. 488 du CNRS, Universite des Sciences et Techniques du Languedoc, Place Eugene Bataillon, 34060 Montpellier Cedex, France*

Received 9 December 1986

A series of 9-β-D-xylofuranosyladenine (xyloA or xyloadenosine) substituted analogs of 2-5A core trimer and tetramer were examined for their ability to be degraded by the 2′,5′-phosphodiesterase activity of cytoplasmic extracts of mouse L cells. Two distinct groups of xyloA-substituted analogs could be readily discriminated. The first group contained xyloadenosine at the 2′-termini and included A2′p5′A2′p5′(xyloA) and A2′p5′A2′p5′A2′p5′(xyloA). These oligomers behaved as did their parent oligoadenylates in that they were equally sensitive to degradation by the 2′,5′-phosphodiesterase activity. The second group of oligonucleotides bore a xyloadenosine residue in the penultimate nucleotide residues of the oligomers and included A2′p5′(xyloA)2′p5′(xyloA), (xyloA)2′p5′(xyloA)2′p5′(xyloA), A2′p5′A2′p5′(xyloA)2′p5′(xyloA) and (xyloA)2′p5′ (xyloA)2′p5′(xyloA)2′p5′(xyloA). This group was quite resistant to 2′,5′-phosphodiesterase activity. In all, the findings demonstrate that the *ribo* configuration 3′-hydroxyl group in the penultimate nucleotide of the oligonucleotide substrate is a prerequisite for the 2′,5′-phosphodiesterase activity.

## 1. INTRODUCTION

The unique 2′,5′-linked oligonucleotide, 2-5A (ppp5′A2′p5′A2′p5′A), is synthesized in vivo and in vitro by the interferon-induced enzyme 2-5A synthetase, it subsequently may activate the 2-5A-dependent endonuclease, RNase L and it can be degraded to ATP and AMP by a 2′,5′-phosphodiesterase (reviewed in [1–3]). Since this latter degradation enzyme is important in the control of the biological activity of 2-5A and in limiting the activity of 2-5A analogs which may serve as potential chemotherapeutic agents, an understanding of the oligonucleotide structural requirements of and mechanism of action of this 2′,5′-phosphodiesterase activity may be considered paramount.

Correspondence address: P.F. Torrence, Section on Biomedical Chemistry, Laboratory of Analytical Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, US National Institutes of Health, Bethesda, MD 20892, USA

Recently, we reported [4] that degradation by the 2′,5′-phosphodiesterase activity requires a 3′-hydroxyl group on the penultimate nucleotide unit of the 2′,5′-oligonucleotide. Specifically we demonstrated that the 3′-deoxyadenosine substituted 2-5A analog, p5′A2′p5′(3′dA)-2′p5′A, possessed the same high degree of resistance to degradation by the 2′,5′-phosphodiesterase activity as did p5′(3′dA)2′p5′(3′dA)-2′p5′(3′dA) shown [5,6] to be resistant. On the other hand, analogs in which the replacement of the 3′-hydroxyl by hydrogen was effected in the first or third nucleotide residue of p5′A2′p5′-A2′p5′A were much less resistant to degradation [4]. Herein, we examine the 2′,5′-phosphodi-

*Published by Elsevier Science Publishers B.V. (Biomedical Division)*
00145793/87/$3.50 

esterase resistance of a series of 2-5A core trimer and tetramer in which one or more of the adenosine residues have been replaced by 9-β-D-xylofuranosyladenine (xyloadenosine, xyloA).

## 2. EXPERIMENTAL

The preparation of L cell extracts and conditions for protein synthesis have been described [8]. Oligonucleotide degradation was studied under protein synthesis conditions except that amino acids and mRNA were omitted. The final concentrations of components of the reaction mixture were: L cell extract, 20%; KCl, 120 mM; $MgCl_2$, 3 mM; Hepes, pH 7.5, 30 mM; β-mercaptoethanol, 6 mM; creatine phosphokinase, 27 units/ml; ATP, 1 mM; GTP, 0.1 mM; CTP, 0.6 mM; creatine phosphate, 100 mM. Incubations of the specific oligonucleotide in the above system were performed at 30°C with aliquots withdrawn at the indicated times. After the samples were heated at 100°C for 5 min and then centrifuged at 10000 × *g* to remove denatured protein, they were analyzed by HPLC by injection with a standard (2-chloroadenosine). The conditions for HPLC were as follows. A Waters Bondapack C18 column (3.9 × 300 mm) was employed using a Beckman HPLC system with two model 110A pumps and a 421 controller. Detection was with an ISCO model UA-I absorbance/fluorescence monitor. The integration of chromatogram areas was determined using an Altex model C-RIA integrator. Four different elution programs were employed: (i) 0–50% methanol/water (1:1) into ammonium phosphate (50 mM), pH 7, for 30 min; (ii) 0–50% methanol/water (1:1) into ammonium phosphate (50 mM), pH 4, for 30 min; (iii) 0–30% methanol/water (1:1) into ammonium phosphate (50 mM), pH 7, for 20 min then 30–50% methanol/water (1:1) into ammonium phosphate (50 mM), pH 7, for 20 min; (iv) 0–30% methanol/water (1:1) into ammonium phosphate (50 mM), pH 4, over 20 min then 30–50% methanol/water (1:1) into ammonium phosphate (50 mM), pH 4, over 20 min.

The oligonucleotides A2'p5'A2'p5'A and A2'p5'A2'p5'A2'p5'A were obtained as described [9] and the xyloadenosine analogs used in this study were prepared according to published procedures [10].

## 3. RESULTS AND DISCUSSION

The degradation of two distinct series of oligonucleotides was addressed in this study. The first series were trimers based on the trimer A2'p5'A2'p5'A or 2-5A 'core'. In this series, beginning at the 2'-terminus each adenosine residue was replaced to give A2'p5'A2'p5'-(xyloA), A2'p5'(xyloA)2'p5'(xyloA) and (xyloA)2'p5'(xyloA)2'p5'(xyloA) respectively. The second series, based on the tetrameric core A2'p5'A2'p5'A2'p5'A, entailed a similar substitution scheme except that one position isomer was unaccessible; specifically, A2'p5'(xyloA)-2'p5'(xyloA)2'p5'(xyloA). Thus only the degradation rates of A2'p5'A2'p5'A2'p5'(xyloA), A2'p5'A2'p5'(xyloA)2'p5'(xyloA) and (xyloA)-2'p5'(xyloA)2'p5'(xyloA)2'p5'(xyloA) were investigated. Results for the degradation of the trimer series are presented in fig.1 and those for the degradation of the tetramer series are presented in fig.2. Immediately obvious from inspection of figs 1 and 2 is that there is a tremendous dichotomy in behavior between two distinct groups of oligonucleotide trimers or tetramers. The first group, those that were rapidly degraded by the phosphodiesterase activity, included A2'p5'-A2'p5'A, A2'p5'A2'p5'(xyloA), A2'p5'A2'-p5'A2'p5'A and A2'p5'A2'p5'A2'p5'(xyloA). The half-lives for the oligoadenylate cores were approximately 45 min under these particular conditions. The second group, those that were quite resistant to the action of the phosphodiesterase, included A2'p5'(xyloA)2'p5'(xyloA), (xyloA)2'-p5'(xyloA)2'p5'(xyloA), A2'p5'A2'p5'(xyloA)-2'p5'(xyloA) and (xyloA)2'p5'(xyloA)2'p5'-(xyloA)2'p5'(xyloA). The half-life of this latter group greatly exceeded 2 h. Thus, any analog with a xyloA nucleoside unit in the penultimate position of the oligonucleotide was resistant to degradation by the 2',5'-phosphodiesterase. A simple substitution of xyloA at the 2'-terminus was not sufficient to induce resistance; in fact, such analogs had no more stability to degradation than the parent 2-5A core trimer or tetramer.

The results obtained herein are in agreement with our earlier findings [4] that substitution by

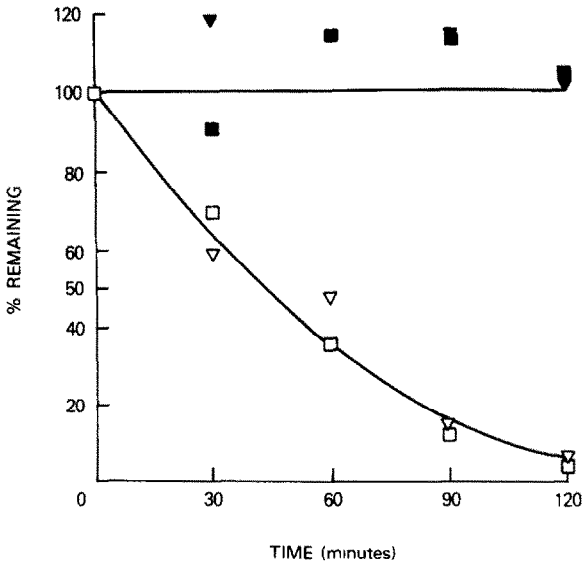


Fig.1. Degradation of various oligoadenylate trimers substituted with xyloA by the 2',5'-phosphodiesterase activity of mouse L cells. Reaction mixtures were made up as described and were incubated at 30°C. At the indicated times, aliquots were withdrawn and analyzed by HPLC. Initial concentrations of the oligomers were $5 \times 10^{-5}$ M. □, A2'p5'A2'p5'A; ▽, A2'p5'A2'-p5'(xyloA); ▼, A2'p5'(xyloA)2'p5'(xyloA); ■, (xyloA)2'p5'(xyloA)2'p5'(xyloA).

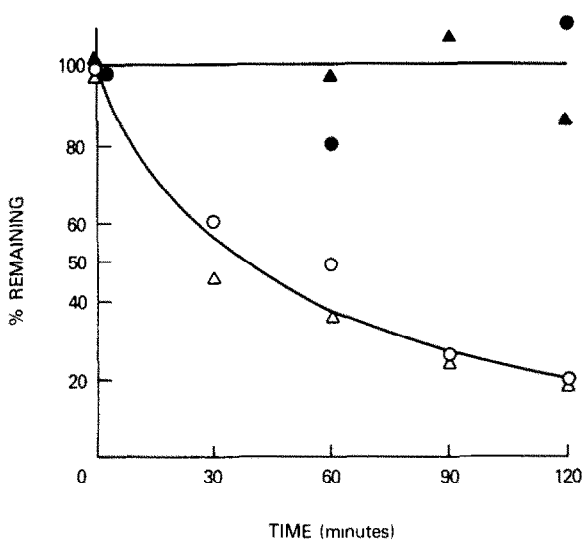


Fig.2. Degradation of various oligoadenylate tetramers substituted with xyloA by the 2',5'-phosphodiesterase activity of mouse L cells. For details, consult the experimental section and the legend to fig.1. Compounds studied were as follows: ○, A2'p5'A2'p5'A2'-p5'A; △, A2'p5'A2'p5'A2'p5'(xyloA); ▲, A2'p5'-A2'p5'(xyloA)2'p5'(xyloA); ●, (xyloA)2'p5'(xyloA)-2'p5'(xyloA)2'p5'(xyloA).

hydrogen of the 3'-hydroxyl group of the penultimate adenosine residue of p5'A2'p5'A2'-p5'A led to resistance to 2',5'-phosphodiesterase activity. The present results also extend this observation to include the generality that the inversion of configuration from *ribo* to *xylo* of the 3'-hydroxyl moiety of the penultimate AMP unit of a 2',5'-oligoadenylate effects a dramatic increase in 2',5'-phosphodiesterase resistance. Of particular interest was the observation that the 2-5A core analogs terminating in xyloadenosine residues were no more resistant to degradation than the parent oligoadenylate. This, coupled with the observation that the 3'-deoxyadenosine substituted analog p5'A2'p5'A2'p5'(3'dA) was degraded only slightly less quickly than the parent triadenylate, makes it improbable that the 3'-hydroxyl group of the 2'-terminal residue of the 2,5'-linked oligomer is a critical recognition point for the 2',5'-phosphodiesterase. That the 3'-hydroxyl moiety of the penultimate nucleotide serves such a critical role in the action of the 2',5'-phosphodiesterase may not be considered unexpected since it is the peculiar stereochemical relationships of the 3'-hydroxyl, the 2',5'-phosphodiester linkage and the remainder of the molecule that would serve to unambiguously identify such an oligonucleotide whereas such recognition information would be lacking in the 2-terminal unit. It remains to be determined whether or not this requirement for a [*ribo*-3']hydroxyl configuration is due to a specific recognition point on the 2',5'-phosphodiesterase for the hydroxyl group, the requisite formation of a 2',3'-cyclic phosphate intermediate of degradation, or both.

## REFERENCES

[1] Torrence, P.F. (1985) in: Biological Response Modifiers – New Approaches To Disease Intervention (Torrence, P.F. ed.) pp.77–105, Academic Press, New York.

[2] Johnston, M.I. and Torrence, P.F. (1984) in: Interferon, Vol.3. Mechanism of Production and Action (Friedman, R.M. ed.) pp.189–298, Elsevier, Amsterdam, New York.
[3] Torrence, P.F., Imai, J., Jamoulle, J.-C. and Lesiak, K. (1986) Chem. Scripta 26, 191–197.
[4] Alster, D., Brozda, D., Kitade, Y., Wang, A., Charubala, R., Pfleiderer, W. and Torrence, P.F. (1986) Biochem. Biophys. Res. Commun., in press.
[5] Sawai, H., Imai, J., Lesiak, K., Johnston, M.I. and Torrence, P.F. (1983) J. Biol. Chem. 258, 1671–1677.
[6] Eppstein, D.A., Marsh, Y.V., Schryver, B.B., Larsen, M.A., Barnett, J.W., Verheyden, J.P.H. and Prisbe, E.J. (1982) J. Biol. Chem. 257, 13390–13397.
[7] Doetsch, P.W., Suhadolnik, R.I., Sawada, Y., Mosca, J.D., Flick, M.B., Reichenbach, N.L., Dang, A.Q., Wu, J.M., Charubala, R., Pfleiderer, W. and Henderson, E.E. (1981) Proc. Natl. Acad. Sci. USA 78, 6699–6703.
[8] Torrence, P.F. and Friedman, R.M. (1979) J. Biol. Chem. 254, 1259–1267.
[9] Torrence, P.F., Imai, J., Lesiak, K., Warinnier, J., Balzarini, J. and De Clercq, E. (1983) J. Med. Chem. 26, 1674–1678.
[10] Huss, S., Gosselin, G., Pompon, A. and Imbach, J.-L. (1986) Nucleosides and Nucleotides 5, 275–300.