Stability of (2′–5′)oligoriboadenylates in various sera

Š. Vilček, O.J. Vrtiak, J. Smrt+ and M. Jurovčík*

Department of Infectology, University of Veterinary Medicine, Komenského 73, CS-04000 Košice, +Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 16610 Prague, and **Institute of Molecular Genetics, Czechoslovak Academy of Sciences, 16637 Prague 6, Czechoslovakia*

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Avian and mammalian sera were found to contain an enzyme activity degrading 2–5A oligonucleotides. The most extensive degradation of the A2′p5′A was observed in chicken serum. Degradation of this compound is not affected by the presence of cAMP, dsRNA, Mg^{2+}, but is significantly inhibited by EDTA. The enzyme activity described is not inactivated by heating to 56°C for 30 min. The 5-mU3′p5′A has also been degraded in chicken serum

(2′–5′)Oligoriboadenylate *Serum* *Phosphodiesterase* *Interferon action*

1. INTRODUCTION

(2′–5′)Oligoriboadenylates were discovered during study of the antiviral effects of interferon [1]. These compounds are synthesized in the cells by the 2–5A synthetase, which after treatment with interferon in the presence of double-stranded RNA (dsRNA) polymerizes ATP through (2′–5′)phosphodiester bonds [2] whereby dimers to heptamers and perhaps higher oligonucleotides are formed.

(2′–5′)Oligoriboadenylates are degraded by 2′-phosphodiesterase [3–5]. Degradation of 5′-triphosphorylated oligonucleotides yields adenosine and ATP [3,4], and oligonucleotides devoid of 5′-phosphate groups (so-called oligonucleotide cores) are converted to 5′-AMP and adenosine [4]. The substrate specificity of the enzyme for compounds with (2′–5′)phosphodiester bonds is not complete, since it also cleaves some oligonucleotides with (3′–5′)phosphodiester bonds [3]. The purified enzyme does not degrade deoxyribonucleotides [3].

Although 2–5A synthetase has been found in the serum of both infected and interferon-treated mice [6], the presence of 2′-phosphodiesterase in the serum has not yet been confirmed. Thus far the enzyme has been detected in various mammalian [3–5, 7–9] and avian [10] cell extracts and in nuclei of HeLa cells [11]. Our aim was to verify the stability of the dimer A2′p5′A and trimer A2′p5′A2′p5′A cores in the serum of some mammals and birds. A number of effects on the stability of A2′p5′A in chicken serum were analyzed in some detail.

2. MATERIALS AND METHODS

A2′p5′A and A2′p5′A2′p5′A were chemically synthesized as in [12], the dimer 5-mU3′p5′A as in [13], and d(T3′p5′G3′p5′C3′p5′A) as in [14]. Double-stranded RNA (dsRNA) was prepared from bacteriophage f2 (kindly provided by Dr J. Doskočil, Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague). Sera of mammals and birds were prepared from fresh blood by a standard procedure. Chicken plasma was prepared from citrated blood.

The stability of the oligonucleotides was checked as follows: Briefly, to 0.2 ml of test serum were added 50 μl of 5–10 μM/ml A2′p5′A, A2′p5′A2′p5′A, 5-mU3′p5′A, 0.4 A_{260}

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d(T3'p5'G3'p5'C3'p5'A). To determine their effect on the stability of A2'p5'A in chicken serum, tested compounds were added to the reaction mixture at concentrations indicated in table 2. Reaction mixtures were then incubated at 37°C. At zero time and after 3 h and 6 h of incubation, 25-μl samples were removed and analyzed by descending chromatography on Whatman no.1 paper in 2-propanol:conc. ammonia:water (6:3:1, by vol.). The absorbance of separated spots was measured at 260 nm and the percentage of the added oligonucleotides remaining undegraded was estimated. In parallel, degradation of the oligonucleotides in chicken serum was estimated qualitatively by thin-layer chromatography (TLC) on Silufol UV_{254} silica gel foils (Kavalier, Votice, Czechoslovakia) in a development mixture identical to that used for the paper chromatograms.

3. RESULTS AND DISCUSSION

The sera of chickens, geese, pheasants, turkeys, sheep and cows were investigated for the degradation of the dimer A2'p5'A. Chicken serum shows the most extensive degradation of A2'p5'A (table 1). Degradation is most probably due to the phosphodiesterase activity which at 37°C degrades 60% of A2'p5'A in 6 h. Similar degradation of the dimer was also observed in chicken plasma. In the serum of other species, A2'p5'A was degraded much less because 80–90% of the oligonucleotides remained undegraded.

The effect of some factors on the degradation of A2'p5'A in chicken serum was studied (table 2). Mg^{2+}, cAMP and dsRNA (chicken nos 1–3) had almost no effect (cAMP having a greater effect than the other two) on degradation of the oligonucleotide, nor was phosphodiesterase activity impaired by heat inactivation of the serum (56°C, 30 min, chicken no.5). The dimer A2'p5'A in chicken serum was degraded much less in the

Table 1

Degradation of A2'p5'A in the serum of some birds and mammals

Serum	Undegraded oligonucleotide (% remaining)		
	0 h	3 h	6 h
Chicken	100	60	40
Chicken[a]	100	63	37
Goose	100	95	83
Pheasant	100	84	79
Turkey	100	96	95
Sheep	100	93	90
Cow	100	88	82

[a] Plasma

[A2'p5'A] = 8 μM/ml. The oligonucleotide was dissolved in 0.1 M phosphate buffer (pH 7.1 ± 0.1) and analyzed by paper chromatography on Whatman no.1 paper in 2-propanol:conc. ammonia:water (6:3:1, by vol.)

Table 2

Effect of some factors on degradation of A2'p5'A in chicken serum

Chicken no.	Serum plus compounds	Undegraded oligonucleotide (% remaining after 6 h)
1	A2'p5'A	38
	A2'p5'A + Mg^{2+} (2.5 mM)	36
2	A2'p5'A	60
	A2'p5'A + cAMP (0.05 mM)	63
3	A2'p5'A	36
	A2'p5'A + dsRNA (500 μg/ml)	42
4	A2'p5'A	36
	A2'p5'A + EDTA (5 mM)	77
5	A2'p5'A	43
	A2'p5'A[a]	40
6	A2'p5'A2'p5'A	22
	5-mU3'p5'A	40

[a] Serum was heated to 56°C for 30 min prior to experiment

[A2'p5'A] = 8 μM/ml, [A2'p5'A2'p5'A] = 5 μM/ml, [5-mU3'p5'A] = 10 μM/ml. The compounds were dissolved in 0.1 M phosphate buffer (pH 7.1 ± 0.1) and oligonucleotides were analysed by paper chromatography on Whatman no.1 paper in 2-propanol:conc. ammonia:water (6:3:1, by vol.). Serum was obtained from normal chickens (Slovgal hybrid)

presence of EDTA (36% *vs* 77% of undegraded A2'p5'A, chicken no.4).

The trimer A2'p5'A2'p5'A is also degraded in chicken serum (table 2, chicken no.6). Our results show that the phosphodiesterase activity in chicken serum is not specific since it degrades the compounds with (2'–5')phosphodiester bonds and the dimer 5-mU3'p5'A (table 2, chicken no.6). The possibility cannot be excluded that the enzymatic activity we observed is the combined effect of two enzymes which utilize two different substrates. Using TLC we observed no degradation of the deoxyribotetramer d(T3'p5'G3'p5'C3'p5'A) in chicken serum (not shown).

Comparison of the activities of the phosphodiesterase observed by us in chicken serum and of the purified 2'-phosphodiesterase isolated from interferon-treated mouse L cells by authors in [3] revealed significant differences:

(i) The 2'-phosphodiesterase from mouse L cells degrades A2'p5'A 6-times faster than U3'p5'A [3], whereas both dimers are degraded at about the same rate by the phosphodiesterase from chicken serum;

(ii) Heat-inactivation experiments showed that the two enzymatic activities differ; the 2'-phosphodiesterase from mouse L cells being inactivated by heating to 56–60°C [4] and the phosphodiesterase we observe in chicken serum remaining unchanged in activity after heating to 56°C for 30 min.

The presence of high levels of phosphodiesterase activity, especially in chicken serum, has not previously been described. Its role in the degradation of compounds with (2'–5')phosphodiester bonds remains unknown. Further studies are needed to determine whether this enzymatic activity is also involved in the metabolism of the (2'–5')oligoriboadenylates which may play an important role in the antiviral resistance induced by interferon.

REFERENCES

[1] Hovanessian, A.G. (1979) Differentiation 15, 139–151.
[2] Kerr, I.M. and Brown, R.E. (1978) Proc. Natl. Acad. Sci. USA 75, 256–260.
[3] Schmidt, A., Chernajovsky, Y., Shulman, L., Federman, P., Berissi, H. and Revel, M. (1979) Proc. Natl. Acad. Sci. USA 76, 4788–4792.
[4] Schmidt, A., Zilberstein, A., Shulman, L., Federman, P., Berissi, H. and Revel, M. (1978) FEBS Lett. 95, 257–264.
[5] Kimchi, A., Shulman, L., Schmidt, A., Chernajovsky, Y., Fradin, A. and Revel, M. (1979) Proc. Natl. Acad. Sci. USA 76, 3208–3212.
[6] Krishnan, I. and Baglioni, C. (1980) Nature 285, 485–488.
[7] Verhaegen-Lewalle, M. and Content, J. (1982) Eur. J. Biochem. 126, 639–643.
[8] Williams, B.R.G., Kerr, I.M., Gilbert, C.S., White, C.N. and Ball, L.A. (1978) Eur. J. Biochem. 92, 455–462.
[9] Minks, M.A., Benvin, S., Maroney, P.A. and Baglioni, C. (1979) Nucleic Acids Res. 6, 767–780.
[10] Ball, L.A. (1980) Ann. N.Y. Acad. Sci. 350, 486–496.
[11] Nilsen, T.W., Wood, D.L. and Baglioni, C. (1982) J. Biol. Chem. 257, 1602–1609.
[12] Karpeisky, M.J., Beigelman, L.N., Michailov, S.N., Padyukova, N.S. and Smrt, J. (1982) Coll. Czech. Chem. Commun. 47, 156–166.
[13] Smrt, J., Kemper, W., Caskey, T. and Nirenberg, M. (1970) J. Biol. Chem. 245, 2753.
[14] Smrt, J. (1982) Coll. Czech. Chem. Commun. 47, 2157–2169.