Influence of the xyloadenosine analogue of 2',5'-oligoriboadenylate on poly(A)-specific, 2',5'-oligoriboadenylate degrading 2',3'-exoribonuclease and further enzymes involved in poly(A)(+)mRNA metabolism

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Abstract

The homogeneous poly(A)-specific 2',3'-exoribonuclease from calf thymus gland, which cleaves both 3',5'- and 2',5'-linked oligoriboadenylates, does not degrade (xyloA2'p)2 xyloA, the xylofuranosyladenosine analogue of the 2',5'A core. This oligonucleotide, which is supposed to enter intact cells rapidly, was found to possess an increased stability and an enhanced antiherpesvirus activity compared to the natural (A2'p)2A (Eppstein, D. A., Barnett, J. W., Marsh, Y. V., Gosselin, G. and Imbach, J.-L. (1983) Nature 302, 723-724). The poly(A) anabolic enzyme, poly(A) polymerase (Mn2+-dependent), from the same source, which is initiated by (A3'p)2 A and its higher oligomers, does not accept 2',5'A core and its xyloadenosine analogue as primer. Both oligonucleotides exert no influence on endoribonuclease IV and on the integrity of the poly(A)-ribonucleoprotein complex.

Introduction

Oligoriboadenylates with 2',5'-internucleotide linkages [ppp(A2'p)nA (n = 2 to about 10) or briefly, 2',5'-A] were first found in interferon-treated cells and rabbit reticulocyte lysates (14, 15, 18), and later also in isolated nuclei of untreated HeLa cells (28). They are synthesized from ATP by a 2',5'A synthetase (14) which is inducible by interferon (4) and activated by double-stranded RNA (18). In the 5'-triphosphate (or 5'-diphosphate) stage, these oligoadenylate species are able to activate a latent cellular endonuclease (RNase I or F; 29, 32) which hydrolyzes viral (3, 25) and cellular (32) single-stranded RNA primarily after UA, UG, and UU sequences (10, 37), resulting in inhibition of protein synthesis (18). The unphosphorylated 2',5'-A core analogues, on the other side, were found to cause in synchronized cells inhibition of DNA synthesis (antimitogenic effect) (10). However, the biological activity and the possible pharmacological usefulness of 2',5'-A are limited by the rapid degradation of the oligomers (19, 30) from the 3' terminus by 2',3'-exoribonuclease (24, 31); the latter enzyme was found by us to be also involved in poly(A) metabolism of poly(A)(+)mRNA (22, 23). Thus, attempts have been undertaken to improve the metabolic stability of the natural 2',5'-A molecules by structural modification of the oligomers, especially in the ribose moieties. However, most of the 2',5'-A analogues synthesized displayed only little metabolic stability (16). Besides the cordycepin analogue of 2',5'-A core, the xyloadenosine analogue, (xyloA2'p)2 xyloA, which has been proven most stable and active (8-9), was found to be the most promising compound synthesized.
In the present report, the influence of the xyloadenosine analogue of 2-5A core on protein and enzyme systems, controlling the posttranscriptional net-polyadenylation of poly(A)(+)mRNA, has been investigated.

Materials and methods

Compounds

The materials were obtained as follows: \([\text{3H}]\text{ATP}\) (specific activity, 11 Ci/mmol) and ppp(A2'p)3A3'[\(\text{32p}\])pCp (3,000 Ci/mmol) from The Radiochemical Centre (Amersham, England); \([\text{3H}]\) poly(A) (37.4 mCi/mmole of phosphate) from Miles Laboratories (Slough, England); \([\text{2,8-3H}]\)adenosine (33 Ci/mmole) from Schwarz/Mann (Orangeburg, NY, USA); (A3'p)2A, (A2'p)2A and A2'p5'A from P-L Biochemicals Inc. (Milwaukee, Wis., USA); poly(A) (single-stranded, with an average relative molecular mass of 248,000 as determined by Studier; 33), ribonuclease A (from bovine pancreas, specific activity 50 Kunitz-units/rag) and ribonuclease T1 (from Aspergillus oryzae, specific activity 280,000 Egami-units/mg) from Boehringer (Mannheim, F.R.G.); oligo(dT)-cellulose from Collaborative Research (Waltham, Mass., USA); GF/C filters and DE81 filter discs from Whatman-Hormuth and Vetter (Heidelberg, F.R.G.); nitrocellulose filters (HA; 0.45 μm) from Millipore (Neu-Isenburg, F.R.G.); Aquasol from New England Nuclear (Dreieichenhain, F.R.G.). (XyloA2'p)2xyloA was chemically synthesized (13).

Enzyme preparations

Poly(A)-specific 2',3'-exoribonuclease was purified to homogeneity from calf thymus as described previously (31). Fraction VI, with a specific activity of 40,270 units/mg protein (0.02 mg protein/ml), was used for the studies.

Endoribonuclease IV was isolated according to Müller (21); step 5 with a specific activity of 1,600 units/mg protein (1.2 mg protein/ml) was used.

Poly(A) polymerase (Mn2+-dependent) was prepared from calf thymus as described earlier (35). The experiments were performed with Fraction V, which possesses a specific activity of 1,840 nmol AMP incorporated/30 min/mg protein (7 mg/ml).

Enzyme assays

The poly(A)-specific 2',3'-exoribonuclease activity was determined essentially as described (31) in 100-μl assays consisting of 100 mM Tris-HCl (pH 9.0), 3 mM MgCl₂, 4 mM 2-mercaptoethanol, 10 μg/ml bovine serum albumin and 100 nmol of [\(\text{3H}\)]poly(A) (10⁴ counts min⁻¹ nmol⁻¹) per ml.

The reaction mixture (60 μl) to determine endoribonuclease IV was composed as follows (21): 100 mM Tris-HCl (pH 8.7), 0.5 mM MnCl₂, 0.2 mM dithiothreitol, 10 μg/ml bovine serum albumin, 100 nmol of [\(\text{3H}\)]poly(A) (3 × 10⁴ counts min⁻¹ nmol⁻¹) per ml and 10 μl of enzyme. After incubation for 30 min at 37 °C, the acid-precipitable radioactivity was collected on DE81 filter discs and processed as described (21).

Poly(A) polymerase reactions were carried out at 37 °C for 30 min in the presence of 200 mM Tris-HCl (pH 8.3), 0.5 mM MnCl₂, 4 mM 2-mercaptoethanol, 20 μg/ml bovine serum albumin, 200 μM [\(\text{3H}\)]ATP (14 dis. min⁻¹ pmol⁻¹), 20 μl of enzyme and the indicated amounts of oligo- or polynucleotide primer in a final volume of 100 μl (22). Products formed were detected as acid-insoluble radioactivity using the GF/C filter technique as described previously (7).

RNA preparations

Cultures of 500 ml of logarithmically growing L5178y mouse lymphoma cells (6, 20) at a concentration of 5.2 × 10⁵ cells/ml were incubated in the presence of 10 μCi of [\(\text{3H}\)]Ado/ml (= 0.3 μM) for 2 h. Polysomes were isolated from 10⁸ cells by differential centrifugation (6). The polysomal preparation (3.9 A₂₅⁷ units) contained 4.6 × 10⁷ dpm/A₂₅⁷ unit. Polysomal [\(\text{3H}\)]Ado labelled poly(A)(+)mRNA was obtained from this preparation by affinity chromatography on oligo(dT)-cellulose (2, 6); elution of the bound poly(A)(+)mRNA from the column was performed with 10 mM Tris-HCl (pH 7.5), 10 mM NaCl. The specific activity of poly(A)(+)mRNA was 9.2 × 10⁷ dpm/A₂₅⁷ unit; the amount of RNA was obtained using a value of 24 A₂₆₀ units/mg of RNA (17).

The poly(A) - poly(A)-associated protein complex (= poly(A)-RNP) was isolated by binding of polysomal poly(A)(+)mRNA to oligo(dT)-cellulose followed by digestion with RNase A and RNase T₁.