J. Biosci., Vol. 11, Numbers 1-4, March 1987, pp. 239-244. © Printed in India.

RNase F and 2',5'-oligoA synthetase activities in mice after poly(I). poly(C) administration

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Abstract. The present study demonstrates the presence of considerable levels of 2',5'-oligoA synthetase activity in tissue extracts from mice. The interferon inducer, poly(I).poly(C), induced the synthetase activity in all the tissue extracts *in vivo.* Similarly, a significant amount of endonuclease RNase F activity is found to be present in these tissue extracts. But interferon induction does not seem to have any significant effect on RNase F activity.

Keywords. RNase; 2',5'-oligoA synthetase activity; poly(I).poly(C) administration.

Introduction

2',5'-OligoA synthetase synthesizes the oligonucleotides ppp(A2'p)$_n$A (n = l–4, collectively known as 2–5A) from ATP (Kerr and Brown, 1978). It is demonstrated that the basal level of 2',5'-oligoA synthetase is present in a wide variety of eukaryotic cells. The level of enzyme activity, however, varies in response not only to interferon (IFN) treatment, but also to hormones and the growth state of the cells (Hovanessian *et al.,* 1977; Ball 1979; Kimchi *et al.,* 1979; Minks *et al.,* 1979; Stark *et al.,* 1979; Krishnan and Baglioni, 1980). 2–5A activates a latent 2-5A dependent endonuclease that degrades mRNA (Hovanessian *et al.,* 1979; Clemens and Williams, 1978; Williams *et al.,* 1979; Farrell *et al.,* 1978). Most of the observations made so far with 2',5'-oligoA synthetase and RNase F are in tissue culture systems (Hovanessian *et al.,* 1977, 1979; Ball, 1979; Kimchi *et al.,* 1979; Minks *et al.,* 1979; Stark *et al.,* 1979; Krishnan and Baglioni, 1980; Clemens and Williams, 1978; Williams *et al.,* 1979; Farrell *et al.,* 1978). Thus, it will be of interest to study the effect of IFN-inducer poly(I).poly(C) on these enzyme activmyies in a whole animal such as mouse. The present study demonstrates that a considerable amount of 2',5'-oligoA synthetase and RNase F activities were present in tissues of mice and also shows the induction of 2',5'-oligoA synthetase only after poly(I).poly(C) administration.

Materials and methods

Chemicals

Oligonucleotides, poly(I).poly(C), T4RNA ligase were obtained from P. L. Biochemical Laboratories; 2',5'-ADP sepharose from Pharmacia Fine Chemicals, Upsala, Sweden; [^{32}P]-pCp from ICN; HPLC grade water and methanol were from Fisher Scientific.

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Animals

C57 BL/6N (about 50 days old) mice were injected with 100 μg poly (I). poly (C) intraperitoneally. At different time intervals 4 mice bled by orbital sinus puncture, were killed and the tissue dissected out and kept frozen at –70°C until used. Mock injected animals served as controls.

Preparation of tissue extract

Tissue homogenates were prepared by homogenizing the tissue in lysis buffer (10 mM Tris-HCl, pH 7·5, 1·5 mM $Mg(OAc)_2$, 10 mM KCl and 7 mM β-mercapto ethanol in a motor-driven Potten-Elvjhem homogenizer and centrifuged for 30 min at 20,000 *g*. The supernatant was stored at — 70°C in aliquots.

Synthesis of ppp(Ap)$_3$ A 321 P pCp

High specific activity ppp$(Ap)_3$A[^{32}P]-pCp was synthesized by incubation 200 μCi 5'- [^{32}P] -pap (lyophilized), 5 μl T_4RNA ligase, 25 μl of ligase buffer (100 mM Hepes buffer, pH 7·6, 15 mM $MgCl_2$, 6·6 mM dithiothretol, 5 μM ribosylthymine 5'-triphosphate, 20 % v/v dimethylsulphoxide) and 400 μM of ppp $(Ap)_3$A in a total volume of 50 μl at 4°C for 20 h. The reaction mixture was then heated at 90°C for 5 min and centrifuged for 10 min at 10,000 *g* to remove denatured proteins. The products were further purified by high performance liquid chromatography on μ Bondapack C_{18} column (Water Associates) using a methanol gradient in 50 mM ammonium phosphate buffer, pH 7·0 as described by Brown *et al.* (1981).

RNase F activity by radiobinding assay method

2–10 μl of S–10 extracts containing 10mg/ml of protein were incubated with ppp$(Ap)_3$A [^{32}P]-pCp (3,000 cpm, 2×10^6Ci mon^{-1} approximately) at 0°C as described for the radiobinding assay by Knight *et al.* (1980).

2',5'-OligoA synthetase assay

Tissue extracts (25–100 μl) and serum (50 μl) were treated with 100 μl packed 2',5'-ADP sepharose at 4°C overnight as described by Johnston *et al.* (1980). The resin-bound synthetase was incubated at 30°C in HGII-ATP buffer (20 mM Hepes, pH 7·5, 90 mM KCl, 0·5 mM $Mg(OAc)_2$, 7mM β-mercaptoethanol, 20% v/v glycerol and 5 mM Mg ATP) made 10^{-4}M poly (I), poly (C) for 6h and then centrifuged. The supernatant was heated to 95°C for 5 min and centrifuged. The heat inactivated supernatant was then assayed for its 2–5 A content by radiobinding assay procedure using extracts from Ehrlich ascites tumor cells as described by Knight *et al.* (1980).

One unit of enzyme is defined as the amount of enzyme required to synthesize 1 nmol of 2–5A/h under standard assay conditions. Specific activity of the enzyme is units/mg of protein.

Protein was measured by Bio-Rad protein assay kit using crystalline bovine serum albumin as the standard.

Results and discussion

2',5'-OligoA synthetase activity

The 2',5'-oligoA synthetase activity was measured in the tissue extracts as described in the 'materials and methods' and the results are shown in figure 1. It shows that all the tissue extracts studied contained considerable amounts of 2',5'-oligoA synthetase activity except brain where the enzyme activity could not be detected with the present assay method. Spleen showed the highest activity and the kidney the lowest. However, there was a sharp increase in the enzyme activity including brain after 5 h of intraperitoneal injection of the IFN inducer poly(I).poly(C). But there seems to be a gradual loss in the enzymatic activity in spleen, liver and lung; a rapid decrease in kidney after 25 h of poly(I). poly (C) injection. Brain still showed the elevated level

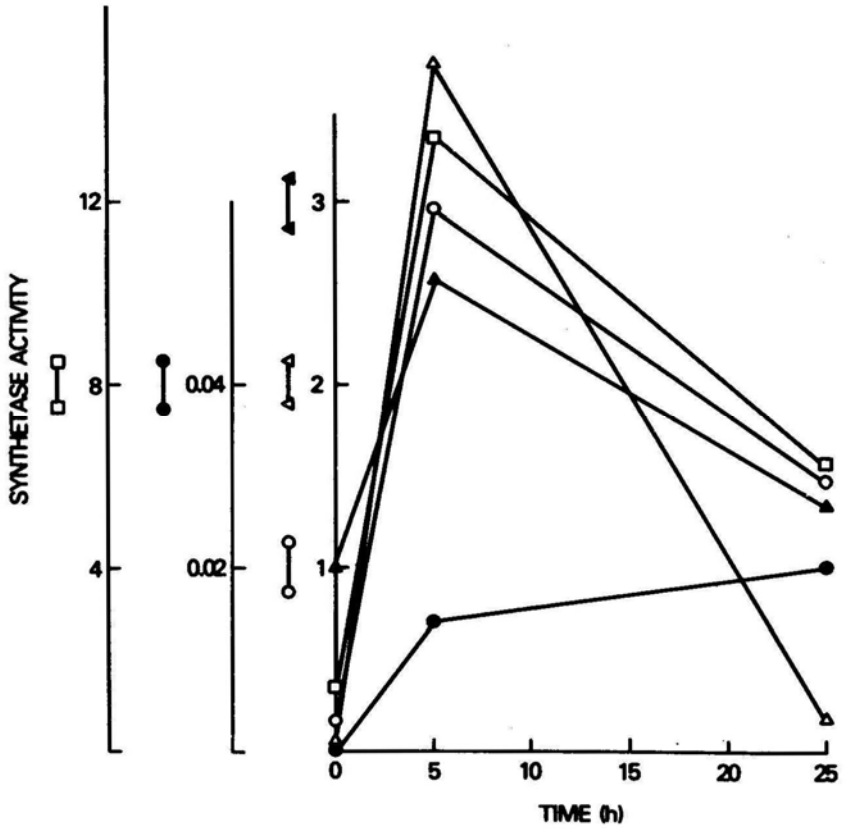

Figure 1. 2',5'OligoA synthetase activity in tissue extracts after poly (I). poly(C) injection. 100 μg poly(I).poly(C) was injected intrapermyoneally and at the time indicated (hours from injection) mice were billed, tissue extracts were made and 2',5'-oligoA synthetase activity was determined as described in the 'materials and methods'. (□) , Spleen; (O), liver; (▲), lung; (Δ), kidney; (●), brain.

of the enzyme activity. Hence, the results indicated that IAN as produced by poly(I). poly(C) injection (Rabinovitch *et al.,* 1977) induced the 2',5'-oligoA synthetase activity similar to that has been well characterized in the IAN-treated tissue culture systems (Kerr and Brown, 1978; Hovanessian *et al.,* 1977, 1979; Ball 1979; Kimchi *et al.,* 1979; Minks *et al.,* 1979; Stark *et al.,* 1979; Krishnan and Baglioni 1980; Clemens and Williams, 1978; Williams *et al.,* 1979; Farrell *et al.,* 1978). The significance of the different level of enzyme activity in different tissues is not clear at present. However, our results are quite compatible with those of Krishnan and Baglioni (1980) with respect to the synthetase activity in spleen and lung. Although they could not detect the enzyme activity in liver and kidney, the reason for this is not clear at present. Moreover, this is the first report of detecting the 2',5'-oligoA synthetase activity in mouse brain.

2 ',5"OligoA synthetase assay was also performed with the serum. But we could not detect the enzyme activity in the serum. This was not unexpected because 2',5'-oligoA synthetase activity had previously been shown only in cell extracts (Baglioni, 1979). However, there is one report on the presence of the synthetase activity in serum (Krishnan and Baglioni, 1980).

Finally, our present results together with other findings on the variations in the level of 2',5'-oligoA synthetase activity in a variety of cell and tissues (Hovanessian *et al.,* 1977; Ball, 1979; Kimchi *et al.,* 1979; Minks *et al.,* 1979; Baksi K., unpublished results), with growth and hormone status (Stark *et al.,* 1979; Krishnan and Baglioni, 1980), its ability to add 5'AMP in 2',5'-linkage to the important metabolites NAD^+, ADP-ribose and A5'P_4 5'A (Ball and White, 1980; Ball, 1980; Ferbus *et al.,* 1981; Minks *et al.,* 1980) implies some role for the 2',5'-oligoA synthetase in regulation of cellular metabolism. Similar conclusion also reached by others (Stark *et al.,* 1979; Ball and White, 1980).

RNase F activity

Tissue extracts are known to contain considerable amounts of RNA degrading enzyme(s) (Slattery *et al.,* 1979), the radiobinding assay procedure was therefore used to assay the RNase F activity in the tissue extracts. Figure 2 shows that all the tissue extracts studied here namely, liver, kidney, lung, spleen and brain contained RNaseF activity. The level of the enzyme activity again varied greatly as also was found with different cell lines (Baksi K., unpublished results). However, the highest activity was observed with spleen and the lowest with liver and brain. Not much information is available on RNase F activity in tissues mainly due to lack of a suitable assay method. Until recently, RNase F activity was assayed by measuring the RNA degradation in presence and absence of 2-5A, therefore, it could only be assayed after purification of the cells and tissue extracts containing high levels of other nucleases (Baglioni *et al.,* 1978; Patner *et al.,* 1978). Nilsen *et al.* (1981) recently have reported the radiobinding assay method of RNase L activity in tissue extracts of rabbit. Furthermore, unlike 2',5'-oligoA synthetase there was not much change in the RNase F activity in the tissue extracts after 5 h from poly (I). poly (C) injection. In all these cases, a 50–60% inhibition of binding was observed with 1 nmol of $ppp(Ap)_2A$ indicating the specificity of the enzyme activity (data not shown).

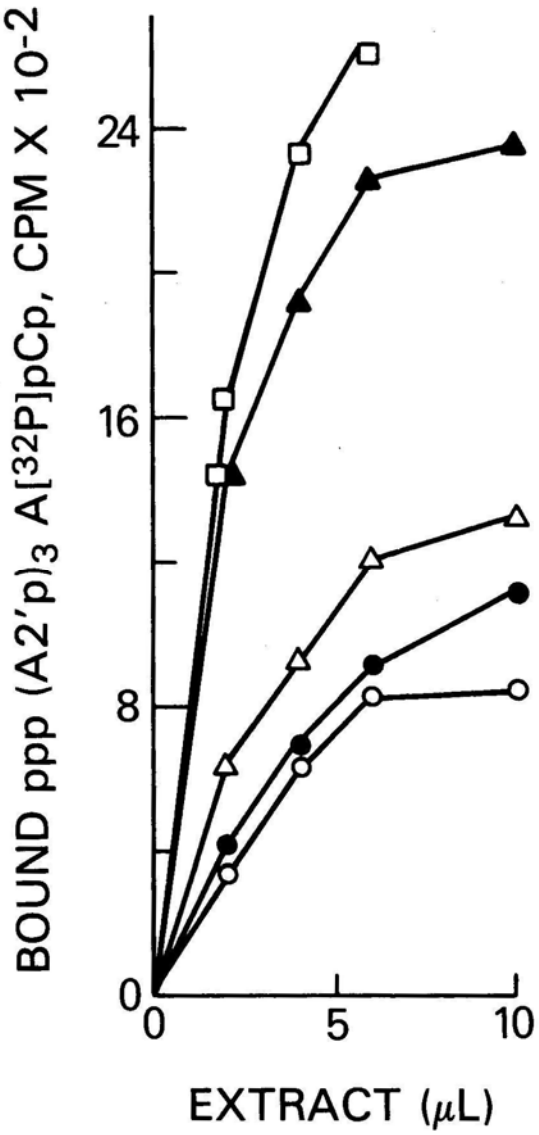

Figure 2. RNase F activity in tissue extracts. RNase activity was determined as described in the 'materials and methods'. (□), Spleen; (○), liver; (▲), lung; (Δ), kidney; (●), brain.

Like the absence of the synthetase activity in the serum, RNase F activity also could not be detected in the serum.

In conclusion, our studies show that tissue extracts of mice contain considerable amounts of RNase F activity; the level of enzyme activity varies in different tissues and IFN does not have much effect on this enzyme activity. Moreover, the present results together with studies by others (Nilsen *et al.,* 1981) also suggest a possible role for the enzyme in the RNA metabolism.

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