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Effect of tryptophan on rat hepatic nuclear poly(A)polymerase activity*

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Summary. Addition of poly(A) to hnRNA in the cell nucleus is a post-transcriptional event and is presumed to be brought about by a specific poly(A)polymerase. Since it is known that tryptophan rapidly increases the cytoplasmic levels of polyadenylated mRNA, it was of interest to investigate whether the essential amino acid, tryptophan, affects the enzyme responsible for polyadenylation. Tryptophan (300 mg/kg body wt.) tube-fed for 10 min elevated the hepatic nuclear enzymatic activities of both the chromatin-bound nuclear poly(A)polymerase (44%, n = 7) as well as that of the free solubilized form (48%, n = 7). Hepatic nuclear proteins separated under denaturing conditions, transferred to nitrocellulose sheets, and then probed with antibody raised against hepatic nuclear poly(A)polymerase showed no differences between the hepatic nuclei of control and tryptophan-treated rats.

Keywords: Amino acids – Poly(A)polymerase – Tryptophan – Rat liver nuclei

Introduction

It has been known for some time that the dietary status may play a role in nucleic acid and protein metabolism. Rats fed a low protein diet reveal decreased activities of hepatic nuclear DNA-dependent RNA polymerases I and II (Anderson and von der Decken, 1975). Also, starvation/fasting not only causes a reduction in the activity of hepatic DNA-dependent RNA polymerases (Henderson, 1970; Vesely and Cihak, 1970) but also causes a decrease both in the level of RNA synthesis (Rickwood and Klemperer, 1970) and in the

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translocation of poly(A)-containing mRNA from nucleus to cytoplasm (Murty et al., 1977). Fasted animals reveal disaggregation of hepatic polyribosomes (Fleck et al., 1965; Sidransky et al., 1968; Sox and Hoagland, 1966; Staehelin et al., 1967; Wilson and Hoagland, 1967; Wunner et al., 1966), which is presumed to be due to decreased activity of poly(A)polymerase (Jacob et al., 1976b) that in turn leads to instability of polyribosomes and hence disaggregation. The decrease in the polyadenylation of mRNA has been demonstrated to be due to an increase in the activity of poly(A)-specific endonuclease without any alteration in the activity of poly(A)polymerase (Matts and Siegel, 1979).

Feeding either a complete amino acid mixture or L-tryptophan alone reverses the changes in RNA metabolism observed in response to starvation/fasting (Fleck et al., 1965; Henderson, 1970; Jacob et al., 1976b; Matts and Siegel, 1979; Murty et al., 1977; Sidransky et al., 1968; Sox and Hoagland, 1966; Staehelin et al., 1967; Vesely and Cihak, 1970; Wilson and Hoagland, 1967; Wunner et al., 1966). Although it has been reported that the essential amino acid, L-tryptophan, has no effect on poly(A)-specific endonuclease (Matts and Siegel, 1979), whether it had an effect on poly(A)polymerase was not known. In this study, we measured the activities of poly(A)polymerase in rat liver nuclei in response to a single feeding of tryptophan. Data are presented which indicate that L-tryptophan rapidly stimulates poly(A)polymerase activity. Using antibodies raised against rat hepatic nuclear poly(A)polymerase, the enzymes of livers of control and tryptophan-treated rats appear to have similar bands on Western Blot analysis.

Materials and methods

Animals

Female rats of the Sprague-Dawley strain (Sprague-Dawley, Madison, WI), weighing on the average 150 g, were used in all experiments. Rats, maintained in an air-conditioned room, were fed a commercial diet (Purina Laboratory Chow # 5001, Purina Mills, Inc., St. Louis, MO) Both control and treated rats were fasted overnight. Some of the fasted rats were tube-fed L-tryptophan (300 mg/kg body weight) in water by stomach tube for 10 min prior to sacrifice (decapitation). Control animals received an equal amount of water by the same route.

Isolation of liver cell nuclei

Rat hepatic nuclei were isolated as described by Blobel and Potter (1966). Briefly, the liver was homogenized in 3 volumes of 0.25 M sucrose, containing 0.5 M Tris HCl pH 7.5, 5 mM $MgCl_2$, and 25 mM KCl (Buffer A), using a motor driven teflon pestle. The sucrose content of the homogenate was raised to 1.62 M by using 2.2 M sucrose in the same buffer. The nuclei were isolated by layering 1 volume of the homogenate over 1 volume of 2.2 M sucrose in Buffer A, followed by centrifugation at 125,000 × g for 1 h.

Enzyme assay

Poly(A)polymerase activity was measured as described by Kurl et al. (1988a, b), with slight modifications. Rat hepatic nuclei (50-100 μg protein) were incubated for 60 min at 37 °C in the presence of ATP (0.5 nmol), Mn^{2+} (1.7 mM), KCl (40 mM), water or poly A (200 μg/ml), ^{3}H-ATP (1.5 μCi), and 0.05 M Tris (pH 8.0) contained in a total volume of 120 μl. The

incubation was terminated by placing the tubes in ice, and aliquots (80 μl) were spotted on DE-81 filter discs (25 mm). The filter discs were washed 4 times with sodium phosphate dibasic, rinsed with distilled water, and counted in the presence of ACS II (Amersham). Since Jacob et al. (1976a) reported that isolated hepatic nuclei contain two distinct physiologically active forms of poly(A)polymerase (chromatin-bound form and free form), we measured for both forms (free determined with added poly A and bound without the addition).

Western Blot analysis

Nuclear proteins were separated on polyacrylamide gels (12%) under denaturing conditions and electrophoretically transferred to nitrocellulose sheets (45 μ). The sheets were blocked with 10% BSA in phosphate buffered saline (PBS) overnight at 4° C and then probed with 1:2000 dilution of the rabbit polyclonal serum raised against the purified rat hepatic nuclear envelope protein with poly(A)polymerase activity as described earlier (Kurl et al., 1988c). The nitrocellulose sheet was washed again and incubated with peroxidase labeled secondary antibody. The antigen-antibody complex was visualized with 0.1 M Tris pH 7.4 containing 0.5 mg/ml of the substrate, 3, 3′-diaminobenzidine and 0.005% (v/v) hydrogen peroxide.

Protein assays

Proteins were precipitated with 10% trichloracetic acid prior to assay according to Lowry et al. (1951).

Results

Initially, experiments were performed in order to optimize the requirements for the measurement of enzymatic activity in rat hepatic nuclei.

Protein

The incorporation of (^{3}H)AMP was directly proportional to the amount of nuclear protein. Enzymatic activity of both the free form (i.e., activity measured in the presence of poly(A)) and the bound form (i.e., activity measured in the absence of poly(A)) increased linearly when 25–100 μg of protein was used (Fig. 1A). During this study, 50–100 μg of protein were used.

Time

The reaction was essentially linear for the time periods (0–60 min) studied (Fig. 1B). The incorporation of (^{3}H)AMP by the free form of the enzyme was higher than that of the bound form.

Concentration of poly(A)

Only the activity of the engaged (bound) form of the enzyme could be measured in the absence of poly(A). Maximal activity of the free form of the enzyme was detectable with as little as about 95 μg/ml of poly(A). At concentrations of higher than 375 μg/ml, the enzymatic activity decreased (Fig. 1C).

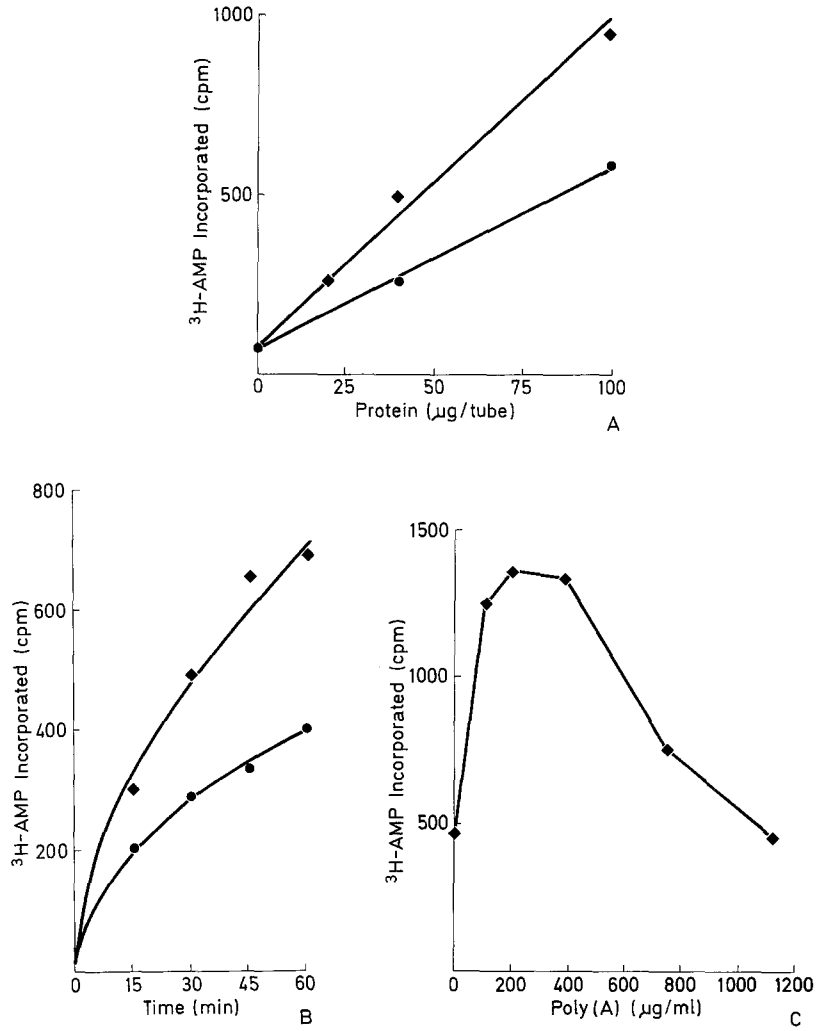

Fig 1. Optimization of the poly(A)polymerase reaction. Hepatic nuclei were incubated at 37° C and (^{3}H)AMP incorporated was determined as described in Materials and methods. Assays were performed with the use of varying (**A**) protein concentrations, (**B**) time, and (**C**) poly(A). ■——■ free form; ●——● bound form

Concentration of Mn^{2+}

Little enzymatic activity was observed in the absence of Mn^{2+}. With increasing concentrations of Mn^{2+} (up to 1.8 mM), the incorporation of (^{3}H)AMP increased (Fig. 2A). At the highest concentration of Mn^{2+}, there was a 30-fold and 6-fold increase in the activity of free and bound forms, respectively.

Concentration of KCl

The absence or presence of KCl did not affect the activity of the engaged form of the enzyme (Fig. 2B). An increase in concentration of KCl enhanced enzymatic activity measured in the presence of poly(A) reaching maximal activity at 37.5

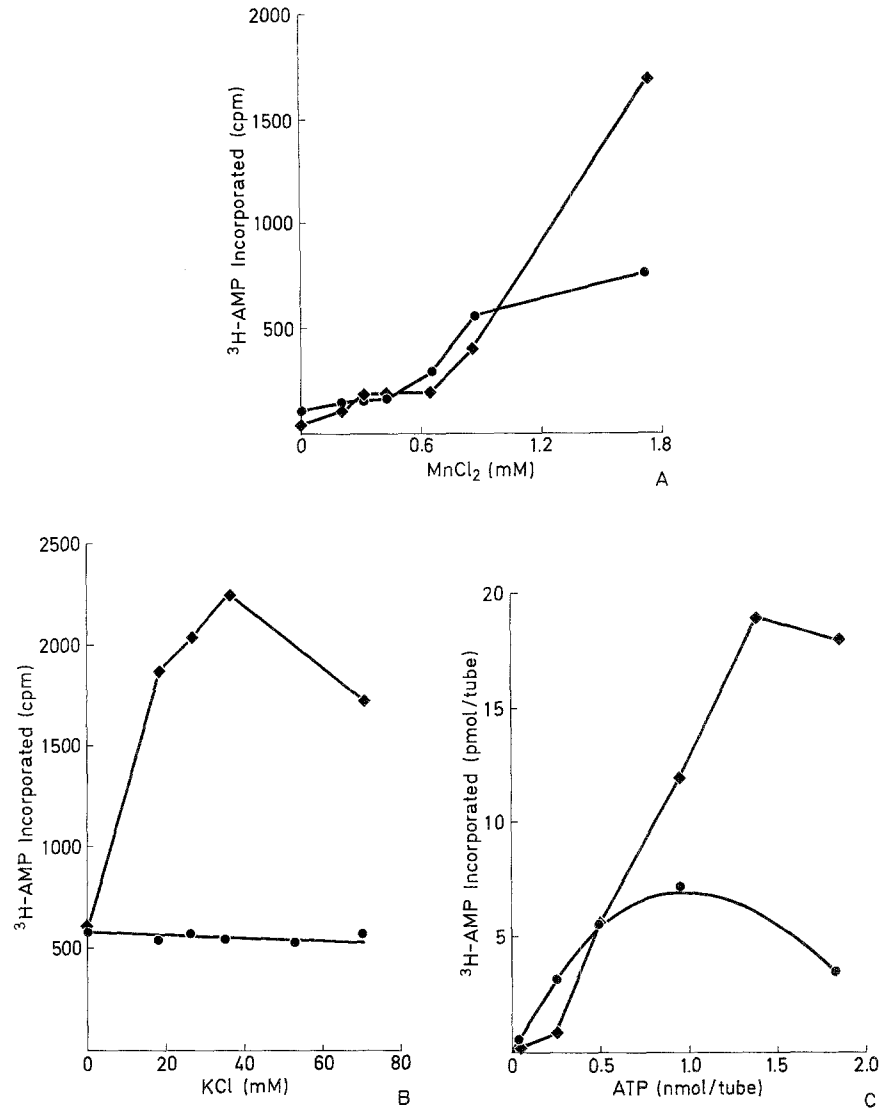

Fig 2. Further optimization of the poly(A)polymerase reaction. Hepatic nuclei were incubated at 37° C for 60 min and (^{3}H)AMP incorporated was determined as described in Materials and methods. Assays were performed with the use of varying (**A**) $MnCl_2$ concentrations, (**B**) KCl concentrations and (**C**) ATP concentrations. ■——■ free form; ●——● bound form

mM KCl. Further increase in salt concentration decreased enzymatic activity (Fig. 2B).

Concentration of ATP

Negligible activity was observed in the absence of ATP. Maximum incorporation of (^{3}H)AMP was observed in the presence of 8.3 nM ATP with respect to the engaged form of the enzyme (Fig. 2C), whereas incorporation of the free form was on the linear scale at this concentration of ATP.

Effect of polynucleotides

To ensure that the enzyme activity was specific for poly(A), assays were performed in which poly(A) was replaced by equal concentrations of poly(U) and poly(G). As shown in Table 1, incorporation of (^{3}H)AMP in the presence of poly(U) and poly(G) was 36% and 43%, respectively, less than with poly(A) (Table 1).

Table 1. Effect of synthetic polynucleotides on poly(A)polymerase activity (free form) of rat hepatic nuclei

Polynucleotide added[a]	Activity (%)
Poly(A)	100[b]
Poly(U)	64
Poly(G)	57

[a] 300 μg/ml of each was added
[b] 100% corresponds to 5194 cpm of ^{3}H-AMP incorporated/mg nuclear protein

Measurement of poly(A)polymerase activity of rat liver nuclei after tube-feeding tryptophan

The effect of tryptophan on hepatic nuclear poly(A)polymerase activity was determined on overnight-fasted rats tube-fed L-tryptophan (300 mg/kg body weight) in water or water alone (controls) for 10 min. This early time interval was selected since earlier experiments have demonstrated that tryptophan stimulates a variety of parameters by 10 min (Sidransky, 1985). Table 2 summarizes the results and reveals that tryptophan increased the enzymatic activity of both the bound and free forms of poly(A)polymerase.

In order to determine whether there was a structural difference between the hepatic nuclear enzymes of the control and tryptophan-treated animals, nuclear

Table 2. Effect of tryptophan on rat hepatic nuclear poly(A)polymerase activity

		Nuclear enzyme activity (%)	
Treatment[a]	No. of experiments	Bound form	Free form
Water	8	100[b]	100[c]
Tryptophan	8	144 $\pm$ 13[d]	148 $\pm$ 17[d]

[a] Animals received intragastrically either the vehicle (water) or tryptophan (300 mg/kg body weight) for 10 min
[b] 100% corresponds to 4698 $\pm$ 451 cpm of ^{3}H-AMP incorporated/mg nuclear protein
[c] 100% corresponds to 6077 $\pm$ 1124 cpm of ^{3}H-AMP incorporated/mg nuclear protein
[d] $0.05 > P > 0.01$

proteins were separated by electrophoresis under denaturing conditions, transferred to nitrocellulose sheets, and the latter were probed with antibody raised against hepatic nuclear poly(A)polymerase. In either case, a band with an estimated molecular weight of about 64,000 was evident, suggesting the lack of structural differences between the two samples (Fig. 3). Though no difference was observed in the molecular weight of the enzymes, it can be argued that a structural change other than alterations in molecular weight could have been possible in response to tryptophan but not evident with the Western Blot technique.

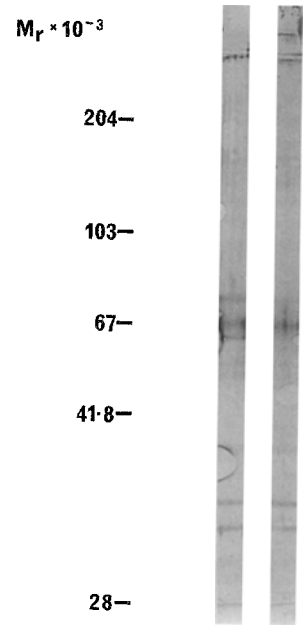

Fig 3. Electrophoresis of hepatic nuclear proteins and then probed with rabbit polyclonal serum raised against rat hepatic nuclear poly(A)polÿerase as described in Materials and methods. Left lane, control; right lane, tryptophan-treated group

Discussion

Though the administration of amino acids has been reported to affect poly(A) polymerase activity (Jacob et al., 1976b), we were not aware that any previous report demonstrated that a single amino acid could stimulate rat hepatic poly(A)polymerase activity. Such an effect was found in this study following the administration of tryptophan by stomach tube to overnight-fasted rats and killed after 10 min. At this selected time, several effects related to RNA metabolism were observed due to tryptophan (Sidransky, 1985). One of the first observations reported 10 min after tryptophan was the ability of rat hepatic nuclei to release in vivo labeled RNA in vitro (Murty et al., 1977; 1979). The increased efflux of RNA was resistant to inhibitors of transcription and translation (Murty et al., 1977). Moreover, there was a concomitant increase in hepatic nucleoside

triphosphatase activity (Murty et al., 1980), which is presumed to play a role in transport of RNA from the nucleus to the cytoplasm (Agutter et al., 1976). It has been reported that the nuclear RNA transported in vitro is polyadenylated and is not due to a shift in intracytoplasmic translocation of polyadenylated mRNA from informosomal pools (Garrett et al., 1984). Thus, it was logical to investigate the effect of tryptophan on hepatic nuclear poly(A) polymerase.

In a recent report, Kurl et al. (1992) presented findings that suggested that the tryptophan receptor (Kurl et al., 1987; 1988c) and poly(A)polymerase (Kurl et al., 1988b) of rat hepatic nuclei share structural homology. This interpretation was based upon data obtained from experiments involving affinity chromatography, immunoblotting, antigen-antibody interaction, and SDS-PAGE. Thus, it cannot be ruled out that the nuclear tryptophan receptor protein and the poly(A)polymerase are parts of the same molecule, and that binding of the amino acid to the receptor site somehow alters the molecular configuration so as to make the enzyme more active.

In a recent report (Sidransky et al., 1990), overnight-fasted rats tube-fed tryptophan for 10 min revealed appreciable binding of tryptophan to hepatic nuclei (less tryptophan binding sites available on hepatic nuclei incubated in vitro with ^{3}H-tryptophan compared to controls). Within 10 min, tube-fed tryptophan caused increased hepatic nuclear poly(A)polymerase activity (Table 1) as well as increased other hepatic nuclear enzyme activities [nucleoside triphosphatase (Murty et al., 1980), protein phosphokinase and phosphoprotein phosphohydrolase (Murty et al., 1983)]. Our current findings that tryptophan administration rapidly stimulates hepatic poly(A)polymerase may indicate that the tryptophan-receptor protein may associate with the poly(A)polymerase into a multimeric species whereby tryptophan binding to its cognate receptor protein enhanced polyadenylation of recently synthesized RNA.

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