Biochimica et Biophysica Acta, 661 (1981) 54–62
Elsevier/North-Holland Biomedical Press

BBA 69348

PURIFICATION AND PROPERTIES OF A POLYADENYLATE POLYMERASE FROM *ARTEMIA* DORMANT EMBRYOS

LEANDRO SASTRE and JESÚS SEBASTIÁN

Instituto de Enzimologia del CSIC, Facultad de Medicina, Universidad Autónoma, Calle Arzobispo Morcillo 4, Madrid 34 (Spain)

(Received February 27th, 1981)

Key words: Poly(A) polymerase; Development; (Artemia embryo)

Soluble extracts from encysted dormant embryos of *Artemia* contain a poly(A) polymerase activity which has been partially purified and characterized. The enzyme requires manganese, an RNA primer and ATP for maximal activity. The K_m for ATP is 0.04 mM and the enzyme is inhibited by concentrations higher than 0.5 mM ATP. dATP replaces ATP with a 15% efficiency. The K_m for dATP is 0.06 mM. Natural RNAs, poly(A) and poly(AG) are the best RNA primers among several homopolymers and copolymers tested. The poly(A) polymerase does not show any specificity for the nucleotide in the 3′ end of the RNA primer. The product of the reaction is a polyadenylic acid chain covalently bound to the RNA primer molecule. The length of the poly(A) chain is about ten nucleotides, but this length is dependent on the incubation time and the RNA primer concentration. The molecular weight of the enzyme is 70 000 and its isoelectric point is 6.0. The existence of an active poly(A) polymerase in dormant embryos of *Artemia*, likely with a cytoplasmic localization, suggests a role for this enzyme in the processing and activation of the stored mRNAs after resumption of development.

Introduction

The embryonic development of *Artemia* has two alternative pathways, the viviparous and oviparous. In the latter case, the zygotes develop inside the ovisac until the gastrula stage. The embryos, which are then covered with a thick shell, are released to the environment, where they undergo a dehydration process. This developmental pathway of *Artemia* involves a cryptobiotic period. The encysted dormant embryos, which are viable for long periods of time, resume their development giving rise to free swimming larvae (nauplii) in about 16–18 h under suitable conditions of rehydration, temperature and oxygenation.

Artemia dormant embryos contain the enzymatic machinery and factors for the transcription [1] and translation [2] of the genetic information, as well as stored messenger RNAs [3,4] which contain short poly(A) tails [5] and caps [6]. *Artemia* is an interesting biological model system to study the mechanisms involved in the transition from a metabolically dormant stage to an active developing one. In particular, *Artemia* cysts are suitable to study the mobilization and activation of the stored informational molecules such as mRNAs. The maturation of the mRNAs in eukaryotic cells involves a complex pathway which occurs in the nucleus and the cytoplasm [7]. The post-transcriptional addition of a poly(A) tail covalently bound to the 3′ end of the RNAs is one of the events that takes place during the processing of most of the messenger RNAs [8]. The biological role of this poly(A) chain is at present not entirely known, However, polyadenylation of stored mRNAs has been described after fertilization or resumption of development in sea urchin [9,11] and plant seeds [12]. These results could indicate that polyadenylation is an important step in the activation of the stored mRNAs in developing systems.

We have undertaken the investigation of the mechanisms involved in the activation of the mRNAs present in *Artemia* dormant embryos and the presence of the enzymes implicated in mRNA proces-

sing. In this paper we report the partial purification and characterization of a poly(A) polymerase from *Artemia* cysts. The poly(A) polymerases are the enzymes that have been implicated in the polyadenylation of the mRNA molecules [13].

Materials and Methods

Materials. Nucleotides, ATP-agarose, homopolymers and heteropolymers were obtained from Sigma Chemical Co. DEAE-Sephadex A-25, Sephadex G-150, poly(A)-Sepharose, poly(U)-Sepharose and ampholites were obtained from Pharmacia. *Torula* RNA, pancreatic RNAase and T_1 RNAase were purchased from Calbiochem. DEAE-cellulose was purchased from Serva. Bio-Rex 70 was obtained from Bio-Rad. PEI cellulose was purchased from Macherey Nagel Co. Calf thymus DNA was obtained from Worthington and formamide from Merck. Glass fibre filters were obtained from Whatman. ^{3}H-Labelled nucleotides, [^{14}C]ITP and [α-^{32}P]ATP were purchased from the Radiochemical Centre, Amersham.

Artemia cysts were obtained from San Francisco Bay Brand Inc., Division of Metaframe Co., Menlo Park, CA 94 025, U.S.A.

Artemia and *S. cerevisiae* ribosomal RNA were extracted from the ribosomal pellets by a phenol-sodium dodecyl sulfate (SDS) treatment. The ribosomal RNA species were separated by sucrose density centrifugation. ^{32}P-labelled yeast ribosomal RNA was prepared by in vivo labelling with ^{32}P [14]. The poly(U)-Sepharose fraction of the *Artemia* RNAase was prepared according to Sebastian and Heredia [15].

Buffer L contains: 50 mM Tris-HCl/0.4 mM EDTA/5 mM mercaptoethanol/20% glycerol, pH 8.5.

Treatment of Artemia cysts. Artemia dry cysts were resuspended in ice-cold distilled water and after 1 h the floating cysts were discarded and the sedimenting embryos were washed twice with distilled water. The cysts were further treated with 0.1% sodium hypochlorite for 10 min and washed several times with cold distilled water. The hydrated cysts were collected by filtration on a cloth.

Enzymatic assays. The standard mixture for the poly(A) polymerase assay contained in a volume of 0.15 ml: 50 mM Tris-HCl, pH 8.5/2 mM $MnCl_2$/0.12 mM ATP/1 μCi [8-^{3}H]ATP/0.2 mg RNA primer/enzyme preparation. The specific activity of the ATP was 30 cpm/pmol. Incubation was for 30 min at 30°C. The reaction was stopped by addition of 5 ml ice-cold 5% trichloroacetic acid/2% tetrasodium pyrophosphate. The trichloroacetic acid-insoluble material was collected on Whatman glass fibre filters, washed, dried and counted in a toluene based scintillation fluid in a liquid scintillation counter. One unit of poly(A) polymerase activity was defined as the amount of enzyme that catalyzes the incorporation of 1 nmol AMP (from ATP) into trichloroacetic acid insoluble material, in 60 min under the assay conditions.

Adenosine triphosphatase activity was determined by the method described by Serrano [16].

Ribonuclease activity was measured following methods as described previously [15].

Separation of nucleotides and adenosine after alkaline hydrolysis of the RNA. The product of the poly(A) polymerase reaction was purified by filtration on Sephadex G-25 and precipitated with ethanol. The RNA pellet was resuspended in 0.3 M KOH and incubated overnight at 37°C. The separation of AMP and adenosine for their quantitative determination was carried out by TLC on polyethyleneimine (PEI)-cellulose following a modification of the method of Reyes [17]. After application of the sample, the plate was first developed with water. The adenosine migrated with the front and the phosphorylated nucleosides remained in the origin. After drying, the plate was developed with monopotassium phosphate (pH 3.5) to separate AMP from contaminating ATP in the assay mixture. The spots were localized with ultraviolet light. Quantification of the labelled AMP and adenosine was done by cutting the plate in 1 cm × 3 mm strips and radioactivity was determined by liquid scintillation counting.

The separation of nucleoside monophosphates, resulting from the alkaline hydrolysis of the ^{32}P-labelled RNA product of poly(A) polymerase, was carried out by high voltage electrophoresis in Whatman 3 MM paper following the method of Smith [18]. The buffer was 0.5% pyridine/0.5% acetic acid/1 mM EDTA, pH 3.5. The electrophoresis was done at 40 V/cm during 2.5 h. After electrophoresis the paper was dried and cut into strips of 1 × 2 cm and radioactivity determined by liquid scintillation

counting. Standard mononucleotides were run in parallel.

Determination of the length of the poly(A) chain. The determination of the poly(A) size was carried out following the method described by Winters and Edmonds [19]. The purification of the ^{3}H-labelled product, as well as its alkaline hydrolysis and fractionation of AMP and adenosine, were done as previously described. The ratio of radioactivity in AMP + adenosine/adenosine was used as a measure of the average chain length of the poly(A) product, since each poly(A) chain gives rise to one adenosine molecule after alkaline hydrolysis.

Protein determination. Proteins were assayed by the method of Lowry et al. [20] using bovine serum albumin as standard.

Results

Partial purification of a poly(A) polymerase from Artemia dormant embryos

Soluble extracts from *Artemia* dormant embryos contain an enzymatic activity dependent on the presence of ATP, manganese and RNA for maximal activity. The product of the reaction can be precipitated by trichloroacetic acid. The requirements of this enzymatic activity were similar to those of the poly(A) polymerases from other biological systems [13]. The following purification procedure was developed in order to characterize the enzymatic and molecular properties of this activity.

Step 1. Preparation of the soluble extract. 30 g hydrated *Artemia* dormant embryos were homogenized with 2.5 vol. buffer L in a Kontes-Dual grinder at 400 rev./min for 5–10 min. This and all subsequent steps were done at 4°C. The homogenate was centrifuged at 10 000 × *g* for 30 min. The supernatant was centrifuged again at 105 000 × *g* for 120 min and the resulting supernatant fraction was the soluble extract.

Step 2. DEAE-cellulose chromatography. A column of 70 ml DEAE-cellulose equilibrated with buffer L/10 mM KCl was loaded with 80 ml soluble extract. After loading the sample, the column was washed with 25 ml buffer L/10 mM KCl and eluted with a linear gradient of 75 ml × 2 buffer L containing 10 mM and 0.5 M KCl. The poly(A) polymerase activity eluted in a single peak at 80 mM KCl. After this step the activity became fully dependent on the presence of RNA in the assay mixture, since the endogenous RNA remains bound to the column. No other peaks of poly(A) polymerase activity were eluted from the column at concentrations of KCl up to 1.0 M. Also only one peak of poly(A) polymerase activity was found using DEAE-Sephadex instead of DEAE-cellulose. The activity recovered after this step was slightly higher than 100%, and the specific activity increased 3-fold.

Step 3. Bio-Rex chromatography. The fractions which contained the activity from the DEAE-cellulose column were pooled and diluted with 1 vol. buffer L. The pool was loaded into a 50 ml Bio-Rex 70 column equilibrated with the same buffer. After loading the sample, the column was washed with 25 ml buffer L/10 mM KCl and eluted with a linear gradient of 75 ml × 2 buffer L containing 10 mM and 0.5 M KCl. The enzymatic activity eluted at 0.2 KCl in a single peak which represented 80% recovery.

Step 4. $(NH_4)_2SO_4$ fractionation. The fractions containing the poly(A) polymerase eluted from the Bio-Rex column were pooled and solid $(NH_4)_2SO_4$ was added to reach a 30% saturation. The solution was kept at 4°C for 1 h and then centrifuged at 10 000 × *g* for 15 min. The resulting supernatant fraction was adjusted to 45% saturation with $(NH_4)_2SO_4$ and kept at 4°C for 1 h. After centrifugation at 10 000 × *g* for 10 min, the resulting pellet was resuspended in buffer L without glycerol. The 30–45% $(NH_4)_2SO_4$ cut contained 60% of the poly(A) polymerase activity.

Step 5. Filtration on Sephadex G-150. The 30–45% $(NH_4)_2SO_4$ cut was filtered through a 90 × 1.5 cm Sephadex G-150 column equilibrated with buffer L without glycerol. The flow rate of the column was 20 ml/h and 2.5-ml fractions were collected. The poly(A) polymerase activity was obtained in a single peak separated from the main protein peak. This step resulted in an 8-fold purification with about 80% recovery. The enzyme was subsequently concentrated by precipitation with 80% $(NH_4)_2SO_4$ and resuspended in buffer L without any loss of activity.

Table I shows a summary of the purification procedure. The enzyme was purified about 100-fold with a final recovery of 40% of the enzymatic activity. The concentrated enzyme was stable for several

TABLE I

PURIFICATION OF A POLY(A) POLYMERASE FROM *ARTEMIA* DORMANT EMBRYOS

Step	Volume (ml)	Protein (mg/ml)	Total activity (units x 10^{-3})	Specific activity (units/mg protein)	Yield (%)
1. Soluble fraction	80	20	5.3	3.3	100
2. DEAE-cellulose chromatography	28	20	5.9	10.5	111
3. Bio-Rex chromatography	63	2.4	4.6	30	78
4. $(NH_4)_2SO_4$ precipitation	1	52	2.5	48	42
5. Filtration on Sephadex G-150	12	0.5	2.1	350	36

weeks at −20°C. The specific activity of the partially purified enzyme is 20-times lower than the poly(A) polymerase purified to homogeneity from calf thymus [21], indicating that the *Artemia* enzyme was only partially purified. However, a further purification of the enzyme has been unsuccessful, since the partially purified poly(A) polymerase did not bind to hydroxyapatite, ATP-Sepharose, poly(A)-Sepharose and poly(U)-Sepharose. The partially purified poly(A) polymerase has been tested for contaminating enzymes, especially for ribonuclease and ATPase activities. The results showed that the poly(A) polymerase preparation had undetectable levels of these activities.

Kinetics of the ATP incorporation

The poly(A) polymerase from *Artemia* dormant embryos shows a kinetics feature similar to that of the enzyme purified from calf thymus [21]. In both cases, the reaction has a lag before a constant rate is reached. The lag is of 5–10 min when *Torula* RNA is used as primer and slightly longer with poly(A) as primer. This lag is not abolished by preincubation of the enzyme with ATP or the RNA primer. After this lag, the reaction was linear for at least 90 min. Another feature common to both enzymes was that the AMP incorporation is not strictly proportional to the amount of the enzyme and that the presence of bovine serum albumin does not affect the behavior of the enzyme.

Substrate specificity

Nucleotide specificity. The partially purified enzyme is highly specific for ATP. CTP, GTP, ITP and UTP are not substrates for the enzyme. The enzyme is unable to incorporate CTP even in presence of ATP. These results indicate that the purified enzyme is not a homopolymer polymerase [22] or the RNA terminal nucleotide transferase [23] and that the poly(A) polymerase preparation is not contaminated with these enzymes. dATP but not dGTP served as substrate for the poly(A) polymerase. The activity of the enzyme with dATP is about 15% of that with ATP. This activity was not due to ATP contamination of dATP. Purity of tritium-labelled dATP was tested by chromatography on PEI-cellulose following the method of Randerath [24]. Over 95% of the product was dATP and less than 1% was contaminating ATP.

Fig. 1 shows the effect of the ATP and dATP concentration on the poly(A) polymerase activity. The enzyme is inhibited at concentrations higher than 0.5 mM ATP. A K_m for ATP of 0.04 mM was calculated from the hyperbolic part of the curve. In contrast, the enzyme is not inhibited by dATP. The enzyme shows an hyperbolic saturation curve with a K_m for dATP of 0.06 mM.

Characterization of the inhibition by ATP of the poly(A) polymerase. The effect of the ATP concentration on the poly(A) polymerase activity with yeast RNA as primer has been represented using Hill and Dixon plots in order to obtain some information on the nature of the inhibition by ATP.

Fig. 2 shows the Hill plot. The experimental points are adjusted to a straight line with a regression coefficient of 0.9. The Hill constant obtained is 1, thus indicating that there is no cooperativity. These results are compatible with an inhibition by ATP of a non-competitive or uncompetitive type with a K_i of 1 mM. The results are in agreement with those obtained from the Dixon plot. The experimental

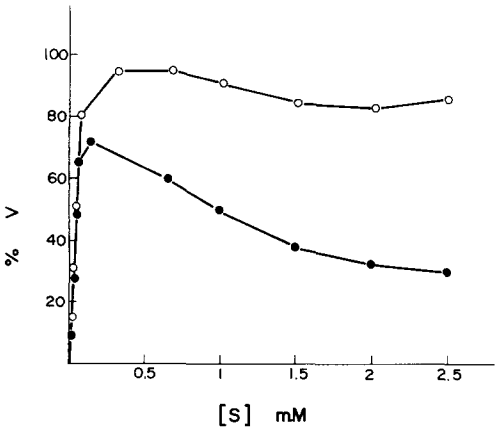

Fig. 1. Effect of ATP and dATP concentrations on poly(A) polymerase activity. Incubations were carried out as described for standard 0.15 ml assay with *Torula* RNA, 2 μCi [^{3}H]NTP, enzyme preparation and ATP (•) or dATP (○) concentrations as indicated. The final $MnCl_2$ concentration in the assay was 2 mM over that of the nucleotide. The V with ATP is 5-times higher than that with dATP.

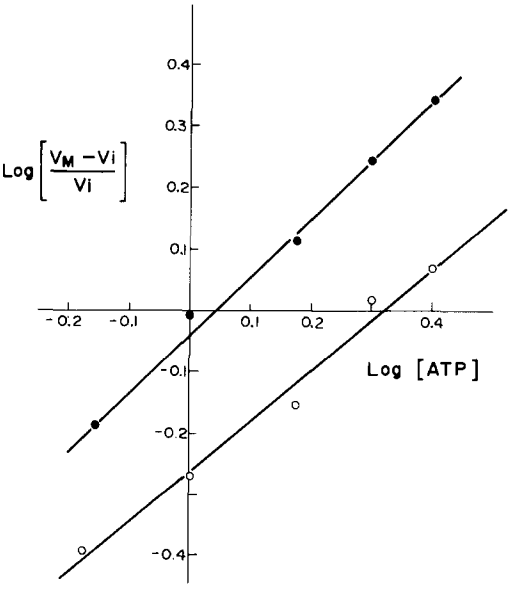

Fig. 2. Hill plot of the inhibition of the poly(A) polymerase by ATP. Determination of the activities of the poly(A) polymerase was carried out with *Torula* RNA (•) and poly(A) (○) as primers and the conditions described for the standard assay.

points are also in a straight line, and thus very close to the theoretical line calculated for a non-competitive or uncompetitive inhibition. The K_i calculated from the Dixon plot is also 1 mM.

The existence of an inhibition of the poly(A) polymerase by excess of substrate implicates the binding to the enzyme of two molecules of ATP. The binding of the two molecules can take place in the same or in different sites of the enzyme. The fact that dATP was also substrate for the poly(A) polymerase and does not inhibit the reaction (Fig. 1) suggests the existence of two sites for ATP, the 'inhibitory site' with no affinity for dATP and the active or 'catalytic site' with affinity for ATP and dATP.

The nature of the presumptive 'ATP inhibitory site' was approached by studying the effect of the ATP concentration on the enzyme activity using poly(A) as RNA primer. In this case, the inhibition is lower than that obtained using yeast RNA as primer, in accordance with the higher affinity of the enzyme for poly(A) (see below). Fig. 2 shows the corresponding Hill plot from which a K_i of 2 mM was calculated. The fact that the K_i for ATP is dependent on the other substrate suggests that the ATP inhibitory site is the same or closely related with the site for the RNA primer. A consequence of this hypothesis is that the inhibition by ATP should be reversed by increasing concentrations of RNA primer. The activity of the poly(A) polymerase was determined at two concentrations of ATP, 0.15 and 1.0 mM, which are non-inhibitory and inhibitory, respectively, and diffferent concentrations of RNA primer. Fig. 3 shows the corresponding double-reciprocal plots. The concentration of ATP changed the K_m of the enzyme for the RNA without affecting the V, indicating the existence of a competition between ATP and the RNA for the primer site.

RNA primer specificity. Table II shows the polynucleotide specificity of the *Artemia* poly(A) polymerase. The poly(A) polymerase activity is maximal with natural RNAs and polyadenylic acid. The activities with other homopolymers are less than 6%. Copolymers were used with different efficiencies and among these, poly(AG), poly(AU) and poly(GU) were the more effective primers. The enzyme is inactive with single- or double-stranded DNA.

The poly(A) polymerase has a hyperbolic satura-

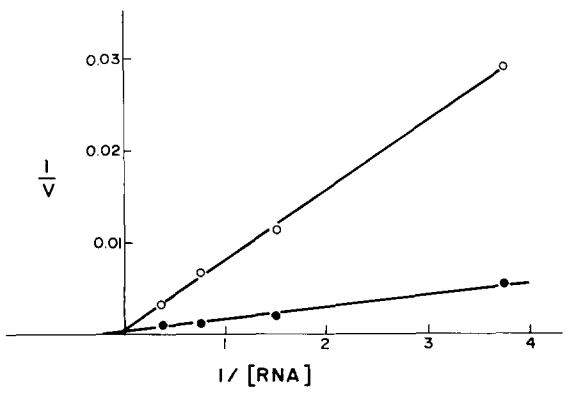

Fig. 3. Lineweaver-Burk plot of the inhibition of the poly(A) polymerase by ATP at different concentrations of RNA primer. The activities of the enzyme were determined with different concentrations of RNA primer, two concentrations of ATP: 0.15 mM (•) and 1 mM (○) and the conditions described for the standard assay.

tion curve with increasing concentrations of poly(A) and yeast RNA. An apparent K_m of 0.01 and 0.03 mM were calculated for poly(A) and yeast RNA, respectively.

Specificity for the 3′ terminal nucleotide of the RNA primer. The higher efficiency of the enzyme with poly(A) as primer when compared with the other homopolyribonucleotides suggested some specificity of the poly(A) polymerase for the 3′ terminal nucleotide of the RNA primer. In order to investigate this property, the enzyme was incubated with yeast RNA using [α-^{32}P]ATP, and the transfer of phosphorus from the AMP residue initiating the poly(A) chain to its nearest neighbour was examined after alkaline hydrolysis of the product and separation of the nucleotides. All four nucleotides were labelled. Therefore, the four nucleotide can be used by the enzyme to start the synthesis of the poly(A) chain. The average chain length of the poly(A) sequence was estimated from the ratio of ^{32}P in AMP to the total ^{32}P in all four nucleotides, assuming that initiation on AMP was an average of the initiation on the other nucleotides. This assumption seems to be correct, since the calculated size of the sequence was the same as that calculated by the ratio adenosine/AMP in an assay mixture containing [^{3}H]ATP as substrate. In both cases the average size of the poly(A) chain using yeast RNA as primer was six nucleotides.

TABLE II

POLYNUCLEOTIDE PRIMER SPECIFICITY OF THE *ARTEMIA* POLY(A) POLYMERASE

Assays were performed as described under "Material and Methods". Polyribonucleotides were present at 1.3 mg/ml and DNA at 0.13 mg/ml.

Primer	pmol AMP incorporated	% Activity
Artemia ribosomal RNA	641	100
Torula RNA	288	45
Poly(A)	270	42
Poly(C)	26	4
Poly(G)	39	6
Poly(U)	6	1
Poly(AG)	256	40
Poly(AU)	122	19
Poly(GU)	147	23
Poly(CU)	32	5
Poly(IC)	13	2
Poly(A) – Poly(U)	51	8
native DNA	3	<1
denatured DNA	3	<1

Divalent cation specificity

The poly(A) polymerase activity is fully dependent on the presence of a divalent cation in the assay mixture. The chloride salts of manganese, magnesium, cobalt, cadmium, calcium, zinc and strontium were tested. The enzyme activity is maximal with manganese, only 7% with magnesium and cobalt, and less than 1% with the other cations. The optimum concentration is between 2–4 mM for $MnCl_2$ under the standard assay conditions for RNA primer and ATP concentrations.

Effect of pH and ionic strength on the enzymatic activity

Maximal activity of the *Artemia* poly(A) polymerase was obtained at pH between 8 and 8.5. At pH 7 the activity is 40% of the maximal. The enzyme is inhibited by low concentration of salt in the assay. 80 mM KCl and 50 mM $(NH_4)_2SO_4$ inhibit 50% of the enzyme activity.

Molecular properties of the Artemia poly(A) polymerase

The poly(A) polymerase is thermosensitive. The

enzyme is stable at 40°C for 5 min, but it is fully inactivated at 50°C. After 5 min at 45°C the enzyme lost 50% of its activity.

The molecular weight of the enzyme was established by gel filtration on a Sephadex G-150 column. The calculated molecular weight of the enzyme is about 70 000.

The isoelectric point of the poly(A) polymerase was determined by electrofocusing in column. The column was loaded with a linear gradient of 5–50% sucrose containing 2.5% ampholites, pH 3–10. The samples was applied in the center of the column. The enzymatic activity was recovered as a single peak, corresponding to an isoelectric point of 6.

Characterization of the reaction product

The nature of the product of the poly(A) polymerase reaction was examined by its ribonuclease sensitivity and its ability to bind the poly(U)-Sepharose. The product of the reaction is insensitive to pancreatic and T_1 ribonucleases and sensitive to the *Artemia* larval ribonuclease [15]. This pattern of sensitivity is the expected one for a polyadenylic acid chain. This conclusion was confirmed after studying the binding of the product to poly(U)-Sepharose. The result of a chromatography of the reaction product on a poly(U)-Sepharose showed that over 90% of the labelled material was bound to the column and was eluted with a buffer containing 90% formamide.

The demonstration of the covalent binding of the poly(A) to the RNA primer was obtained by the ability to transfer the labelled phosphorus from the AMP of the poly(A) chain to the 5′ nearest nucleotide of the RNA primer (the terminal nucleotide) as described in the previous section on the specificity of the 3′ nucleotide.

Size of the poly(A) chain

The size of the poly(A) chain seems to be dependent on the length of the RNA primers. Using RNAs of definite sizes as primers, mainly 26 S, 18 S yeast ribosomal RNAs and 4–5 S *Torula* RNA, resulted in an average size of the poly(A) synthesized by the enzyme of 13, 9 and 6 nucleotides, respectively. The amount of RNA primer used in these assays was the same, but the concentration of free 3′ ends was lower with the higher molecular weight of the RNA primer. Therefore, the length of the poly(A) chain was dependent on the concentration of the RNA primer sequences.

Discussion

The results presented in this paper demonstrate the existence of an active poly(A) polymerase in encysted dormant embryos of *Artemia*. The *Artemia* poly(A) polymerase has been partially purified and some of its molecular and enzymatic properties have been characterized.

Several forms of poly(A) polymerase have been described in animal cells with different chromatographic behavior, subcellular localization and/or enzymatic properties [19,25–33]. However, some of the multiple forms of poly(A) polymerase obtained by ion-exchange chromatography could be due to different states of phosphorylation of the enzyme [34]. The chromatography on DEAE-cellulose of soluble extracts from *Artemia* dormant embryos resolves only one peak of enzymatic activity. The primer and metal specificities of the *Artemia* poly(A) polymerase resemble those described for the cytoplasmic enzyme from animal cells [32,33].

The rate of polymerization of AMP by the *Artemia* poly(A) polymerase has a lag before a linear velocity is reached. This kinetic behavior has also been described for the poly(A) polymerase from calf thymus [21], rat liver [27], hepatomas [30] and sea-urchin embryos [35]. In this respect, Rose and Jacob have found that the initial lag is eliminated by phosphorylation of the enzyme [36].

The study of the nucleotide specificity of the *Artemia* poly(A) polymerase has shown that the enzyme can utilize ATP and dATP as substrates. The apparent K_m for ATP and the activity with dATP are in the same range as those reported for poly(A) polymerase from other organisms [27,28,30,37]. Different experimental evidence indicates that the incorporation of dATP is due to the poly(A) polymerase and not to a DNA polymerase or other contaminating enzyme(s). The incorporation of dATP is dependent on the presence of RNA, but not DNA, in the assay mixture. The ratio of activities with dATP and ATP is constant along the purification steps and there is a competitive inhibition by dATP of the incorporation of ATP and vice versa. Moreover, another deoxynucleotide, dGTP, is not incorporated

by the poly(A) polymerase preparation.

An interesting difference between ATP and dATP is the inhibition of the poly(A) polymerase activity by high concentrations of ATP but not dATP. An inhibition by ATP has also been reported for the nuclear poly(A) polymerase from rat liver [30], but the significance of this inhibition was not further investigated. We have approached the nature of this inhibition and the results are compatible with a competition of ATP with the RNA primer in the interaction of this RNA with the enzyme.

The poly(A) polymerase from *Artemia* dormant embryos requires a polynucleotide for enzymatic activity. Natural RNAs, poly(A) and poly(AG) are the best substrates. DNA and double-stranded RNA are not substrates for the enzyme. The different activities of the poly(A) polymerase with homopolymers and copolymers are not a consequence of a stringent specificity of the enzyme for a particular nucleotide in the 3′ end of the RNA primer, since the enzyme can polyadenylate RNAs with any nucleotide in the 3′ end. The difference can be due to the secondary structure of the synthetic homopolymers and heteropolymers in solution, which will affect their interactions with the enzyme. The ability of the *Artemia* poly(A) polymerase to polyadenylate RNAs as well as poly(A) molecules is compatible with a role in the initiation of the synthesis of the poly(A) chain as well as in the elongation of preexisting polyadenylated molecules.

The existence of an active poly(A) polymerase, probably in the cytoplasmic fraction of *Artemia* dormant embryos, suggests a role for this enzyme in the processing of the RNAs after resumption of development. In this context, it is interesting that sea urchin eggs contain a cytoplasmic poly(A) polymerase, which is supposed to be implicated in the polyadenylation of latent maternal messenger RNAs [35]. In the case of *Artemia*, the enzyme could be involved in the activation of stored messenger RNAs present in dormant embryos [38–41] by elongation of the preexisting polyadenylic chains of these mRNAs [42].

Acknowledgements

We are grateful to Drs. A. Sols, J. Renart and V.D. Villa for reading the manuscript and to Elvira Dominguez for her technical assistance. This work was supported by a grant from "Fondo nacional para el desarrollo de la investigación científica". L.S. has a fellowship from Caja de Ahorros de Madrid.

References

1 Renart, J. and Sebastián, J. (1976) Cell Differ. 5, 97–107
2 Warner, A.H., MacRae, T.H. and Wahba, A.J. (1979) Methods Enzymol. 60, 298–311
3 Clegg, J.S. and Golub, A.L. (1969) Dev. Biol. 19, 178–200
4 Nilsson, M.O. and Hultin, T. (1972) Exp. Cell Res. 72, 145–149
5 Nilsson, M.O. and Hultin, T. (1975) FEBS Lett. 52, 269–272
6 Groner, Y., Grosfeld, H. and Littauer, U.Z. (1976) Eur. J. Biochem. 71, 281–293
7 Darnell, J.E. (1979) Prog. Nucl. Acid Res. Mol. Biol. 22, 327–353
8 Rottman, F.M. (1978) Biochemistry of Nucleic Acids II, Vol. 17, pp. 45–73
9 Slater, I. and Slater, D.W. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 1103–1107
10 Davis, F.C. and Davis, R.W. (1978) Dev. Biol. 66, 86–96
11 Wilt, F.H. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 2345–2349
12 Harris, B. and Dure, L. (1978) Biochemistry 17, 3250–3256
13 Edmonds, M. and Winters, M.A. (1976) Prog. Nucl. Acid Res. Mol. Biol. 17, 149–179
14 Goldberg, S., Oyem, T., Idriss, J.M. and Halvorson, H.O. (1972) Mol. Gen. Genet. 116, 139–157
15 Sebastián, J. and Heredia, C.F. (1978) Eur. J. Biochem. 90, 405–411
16 Serrano, R. (1978) Mol. Cell Biochem. 22, 51–63
17 Reyes, P. (1972) Anal. Biochem. 50, 35–39
18 Smith, J.D. (1967) Methods Enzymol. 12a, 350–361
19 Winters, M.A. and Edmonds, M. (1973) J. Biol. Chem. 248, 4763–4768
20 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193 265–275
21 Winters, M.A. and Edmonds, M. (1973) J. Biol. Chem. 248, 4756–4762
22 Niessing, J. and Sekeris, C.E. (1974) Biochem. Biophys. Res. Commun. 60, 673–680
23 Deutscher, M.P. (1973) Prog. Nucl. Acid Res. Mol. Biol. 13, 51–92
24 Randerath, K. (1963) Biochim. Biophys. Acta 76, 622–624
25 Tsiapalis, C.M., Dorson, J.W. and Bollum, F.J. (1975) J. Biol. Chem. 250, 4486–4496
26 Pellicer, A., Salas, J. and Salas, M.L. (1975) Biochim. Biophys. Acta 378, 107–118
27 Niessing, J. (1975) Eur. J. Biochem. 59, 127–135

28 Rose, K.M., Morris, H.P. and Jacob, S.T. (1975) Biochemistry 14, 1 025–1 032
29 Rose, K.M. and Jacob, S.T. (1976) Biochemistry 15, 5 046–5 052
30 Rose, K.M. and Jacob, S.T. (1976) Eur. J. Biochem. 67, 11–21
31 Hadidi, A. and Sagar-Sethi, V. (1976) Biochim. Biophys. Acta 425, 95–109
32 Nevins, J.R. and Joklik, W.K. (1977) J. Biol. Chem. 252, 6 939–6 947
33 Avramova, Z.V., Milchev, G.I. and Hadjiolov, A.A. (1980) Eur. J. Biochem. 103, 99–107
34 Rose, K.M. and Jacob, S.T. (1979) J. Biol. Chem. 254, 10 256–10 261
35 Slater, D.W., Slater, I. and Bollum, F.J. (1978) Dev. Biol. 63, 94–110
36 Rose, K.M. and Jacob, S.T. (1980) Biochemistry 19, 1 472–1 476
37 Muller, W.E.G., Schroder, H.C., Arendes, J., Steffen, R., Zahn, R.K., and Dose, K. (1977) Eur. J. Biochem. 76, 531–540
38 Nilsson, M.O. and Hultin, T. (1974) Dev. Biol. 38, 138–149
39 Amaldi, P.P., Felicetti, L. and Campioni, N. (1977) Dev. Biol. 59, 49–61
40 Sierra, J.M., Filipowicz, W., and Ochoa, S. (1976) Biochem. Biophys. Res. Commun. 69, 181–189
41 Slegers, H., De Herdt, E., Mettrie, R., Piot, E. and Kondo, M. (1979) in Biochemistry of Artemia development (Bagshaw, J.C. and Warner, A.H., eds.), pp. 84–99, University Microfilm Int., Ann Arbor, MI
42 Sastre, L., Villa, V.D. and Sebastian, J. (1980) Eur. J. Cell Biol. 22, 61