# POLY(ADENYLIC ACID) POLYMERASE: LOSS OF ENZYME FROM RAT LIVER NUCLEI ISOLATED UNDER ISOTONIC CONDITIONS

Kathleen M. ROSE, Yen-Chiu LIN and Samson T. JACOB
*Department of Pharmacology, The Milton S. Hershey Medical Center, The Pennsylvania State University, College of Medicine, Hershey, Pennsylvania 17033 USA*

Received 19 June 1976

## 1. Introduction

Poly(A) polymerase (EC 2.7.7.19), the enzyme which appears to be responsible for the polyadenylation of mRNA, has been detected in several mammalian tissues and is present in nuclear [1–4], mitochondrial [5–7], microsomal [8,9] and cytosol [10,11] fractions. We have recently purified this enzyme to homogeneity from nuclei of rat liver and a rat hepatoma [12] and from mitochondria of a rat hepatoma [13]. Likewise, poly(A) polymerase has been purified from calf thymus tissue [14,15]. Now that the in vitro characteristics of this enzyme have been defined, it is possible to study the response of poly(A) polymerase to various physiological parameters. Indeed, recent studies in our laboratory indicate that nuclear poly(A) polymerase levels appear to be altered in response to amino acid supply [16], glucocorticoid hormone [17] and neoplasia [12]. In order to evaluate quantitatively the levels of enzyme in the nucleus, we determined the effect of different conditions of homogenization of liver tissue on the resultant levels of poly(A) polymerase obtained in the nuclear and cytosol fractions. In this report, we present evidence which indicates that homogenization of liver tissue with isotonic sucrose, a procedure usually employed to obtain the cytosol fraction, results in a loss of almost all the enzyme activity from nuclei. The release of the nuclear enzyme can be minimized by replacing isotonic sucrose with the hypertonic sucrose which is conventionally used for isolation of pure nuclei from mammalian tissues [18,19]. These studies demonstrate the need for the use of hypertonic sucrose in the isolation of nuclei in order to prevent loss of free nuclear enzymes.

## 2. Materials and methods

### 2.1. *Preparation of nuclear and cytoplasmic fractions*

Nuclei and cytosol were prepared from rat liver using hypertonic or isotonic conditions.

#### 2.1.1. a) 'Hypertonic' homogenization

Liver was homogenized in 2.2 M sucrose (7 ml/g liver) containing 15 mM $MgCl_2$ and 0.25 mM spermine essentially as described previously [20]. The homogenate was centrifuged at 40 000 *g* for 75 min to yield the 'hypertonic nuclei'. The supernatant was diluted to 0.44 M sucrose by addition of Buffer I (50 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 25 mM KCl, 0.5 mM dithiothreitol and 0.1 mM EDTA). Mitochondria, lysosomes and microsomes were then removed by centrifugation at 40 000 *g* for 10 min followed by centrifugation at 100 000 *g* for 90 min. The supernatant was designated as 'hypertonic cytosol'.

#### 2.1.2. *'Isotonic' homogenization*

Liver was homogenized in buffer (4 ml/g liver) containing 50 mM Tris-HCl (pH 7.6), 0.25 M sucrose, 25 mM KCl and 10 mM $MgCl_2$. After filtering, the homogenate was centrifuged at 1000 *g* for 7 min. The supernatant was centrifuged again at 1000 *g* for 7 min. The 'isotonic nuclei' from both pellets were combined, then purified by homogenization with 2.2 M sucrose containing 1 mM

$MgCl_2$ (2.5 ml/g liver), followed by centrifugation at 40 000 *g* for 90 min. The supernatant fractions from the 1000 *g* and 40 000 *g* centrifugations were combined, adjusted to 0.44 M sucrose by addition of Buffer I and centrifuged as above to obtain the 'isotonic cytosol'.

### 2.2. *Preparation and assay of poly(A) polymerase*

Poly(A) polymerase was extracted from isolated nuclei as described previously [12,20,21]. Cytosol fractions were precipitated with $(NH_4)_2SO_4$ (0.42 g/ml). After centrifugation the pellets were resuspended and dialyzed overnight against Buffer II (50 mM Tris-HCl (pH 7.9), 25% glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA and 0.5 mM dithiothreitol). Poly(A) polymerase activity was measured using poly(A) as primer as described previously [12]. One unit is equivalent to 1 nmol AMP incorporated per h.

## 3. Results

Poly(A) polymerase was extracted from nuclei prepared by using hypertonic or isotonic sucrose. Enzyme was also obtained from the corresponding cytosol fractions. Because of the presence of nuclease activity which interferes with the poly(A) polymerase assay, the enzyme preparations were subjected to DEAE-Sephadex chromatography. Fig.1 shows the activity profile for the nuclear enzymes. It should be noted that because the 'isotonic nuclei' were further purified through 2.2 M sucrose, the protein and DNA

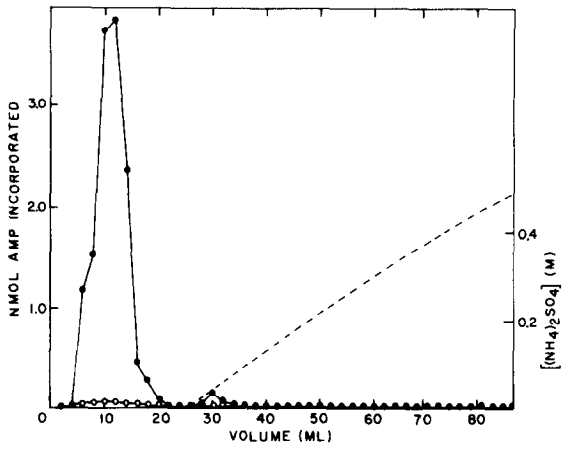


Fig.1. DEAE-Sephadex chromatography of nuclear poly(A) polymerase. Nuclei were prepared from 14 g rat liver using either hypertonic or isotonic sucrose as described in the Methods. Enzymes were extracted and applied to a DEAE-Sephadex column as described previously [21]. The column (0.9 × 8 cm) was washed with Buffer II containing 10 mM $(NH_4)_2SO_4$ and a linear gradient was attached. Aliquots of 30 μl were taken to analyze for poly(A) polymerase activity as described previously [12,16]. (●——●) 'Hypertonic nuclei'. (○——○) 'Isotonic nuclei'.

Table 1
Poly(A) polymerase from nuclei and cytosol

| Preparation | Extract protein (mg) | Column protein (mg) | Total units (nmol/h) | Specific activity (nmol/mg/h) |
|---|---|---|---|---|
| Hypertonic nuclei | 8 | 1.4 | 227 | 176 |
| Isotonic nuclei | 8 | 1.0 | 2 | 2 |
| Hypertonic cytosol | 204 | 94 | 219 | 2 |
| Isotonic cytosol | 200 | 87 | 456 | 5 |

Fractions from individual groups were prepared as described in Methods. Recovery of DNA and protein were identical for both groups of nuclei. 'Extract protein' was determined after $(NH_4)_2SO_4$ precipitation followed by dialysis prior to DEAE-Sephadex chromatography and respresents the total from 14 g liver. 'Column protein' was determined for the pooled fractions after DEAE-Sephadex chromatography following dialysis against buffer containing 50 mM Tris-HCl (pH 8) and 50% (v/v) glycerol. Protein was estimated as described by Bennett [24] using bovine serum albumin as a standard.

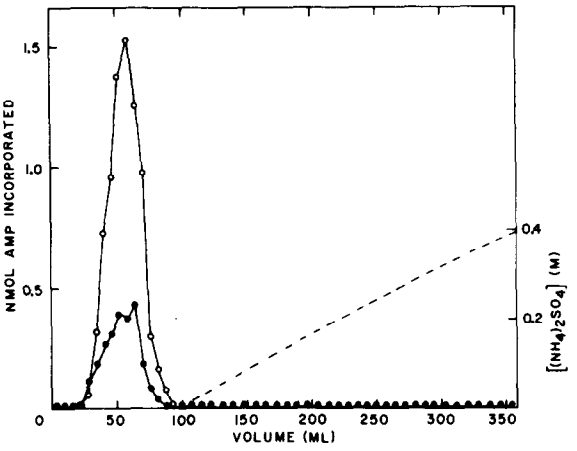


Fig.2. DEAE-Sephadex chromatography of cytosol poly(A) polymerase. Cytosol was prepared concurrently with nuclei from 14 g liver using hypertonic or isotonic sucrose as described in Materials and methods. The dialyzed preparations were applied to 1.5 × 16 cm DEAE-Sephadex column equilibrated in Buffer II. After washing with Buffer II containing 10 mM $(NH_4)_2SO_4$, a linear gradient was attached. Aliquots of 30 μl were taken to analyze for poly(A) polymerase activity. (●——●) 'Hypertonic cytosol'. (○——○) 'Isotonic cytosol'.

content were almost identical to the nuclei prepared by direct homogenization with 2.2 M sucrose, and thus similar amounts of protein were extracted and subjected to ion exchange chromatography (see table 1). As described previously [4,12,16,17,21], an active poly(A) polymerase is obtained from 'hypertonic nuclei'. However, almost no activity was detected in the enzyme extract from 'isotonic nuclei'. Thus, a simple homogenization with 0.25 M sucrose, results in virtually complete loss of poly(A) polymerase from the nuclei. If a transfer of enzyme from nuclei to cytosol has occurred by homogenization in isotonic sucrose, one would expect to find a greater level of enzyme in the 'isotonic cytosol' than in the 'hypertonic cytosol'. As shown in fig.2, 'isotonic cytosol' contained approximately twice as much activity as 'hypertonic cytosol'.

To determine if the increase in total poly(A) polymerase activity in the 'isotonic cytosol' correlated quantitatively with the decrease in activity from the 'isotonic nuclei', fractions from the columns were pooled, dialyzed and assayed using excess poly(A) as primer. As indicated in table 1, the total poly(A) polymerase activity recovered (calculated from the average of several enzyme concentrations) was 227 units in the 'hypertonic nuclei', whereas only 2 units were recovered from the 'isotonic nuclei'. Correspondingly, the poly(A) polymerase activity in the 'hypertonic cytosol' was 219 units compared with 456 units in the 'isotonic cytosol'. A reduction of 225 (227–2) units of poly(A) polymerase activity occurred in nuclei when isolated using isotonic conditions and an increase of 237 (456–219) units of activity was observed in the 'isotonic cytosol' relative to the 'hypertonic cytosol'. Thus, a quantitative transfer of enzyme activity occurs from nuclei to cytosol when the isolation media is changed from hypertonic to isotonic.

## 4. Discussion

We have presented data indicating that the recovery of poly(A) polymerase activity is dramatically altered depending upon the conditions of the isolation. Only 1% of the total nuclear enzyme was retained in 'isotonic nuclei'. This result is consistent with our earlier finding that a large proportion of the nuclear poly(A) polymerase is 'free' in the nucleoplasm and that less than 1% of the nuclear poly(A) polymerase is bound to the chromatin [4]. The 'free' enzyme can be released from the nuclei either by initial homogenization of the tissue with isotonic sucrose or by rehomogenization of the 'hypertonic nuclei' with isotonic media. It is thus evident that measurements of levels of nuclear poly(A) polymerase can only be undertaken using nuclei isolated in hypertonic sucrose. 'Isotonic nuclei' are useful therefore largely in determining the amount of chromatin-bound enzyme. In addition, the most commonly used methods (isotonic) of preparing the cytosol fraction yield an overestimate of enzyme in that fraction due to nuclear leakage.

The data in the present report do not rule out the existence of a separate poly(A) polymerase in the cytosol fraction. The presence of a cytoplasmic poly(A) polymerase is certainly conceivable in view of the additional polyadenylation of mRNA known to occur in the cytoplasm [22,23]. However,

since 2.2 M sucrose is an aqueous medium, albeit hypertonic, it is possible that even some of the poly-(A) polymerase observed in the 'hypertonic cytosol' might be due to nuclear leakage. Assuming the data from the 'hypertonic cytosol' does represent an accurate estimation of enzyme activity, a pattern of enzyme distribution in the liver cell can now be presented. Based on the data in this report for 'hypertonic cytosol' and 'nuclei' together with our previous data for poly(A) polymerase in the other organelles (following one ion exchange chromatography, see [4,7,9,12,21]), we find the following approximate ratio exists for poly(A) polymerase activity in the liver cell; nuclear sap*: chromatin**: mitochondria**: membrane-bound ribosomes**: cytosol as 100:1:3:25:100. It is not yet known whether the enzymes in the different cellular fractions are identical.

It should be noted that the specific activity of the poly(A) polymerase in the 'hypertonic nuclei' is 75 times greater than in the 'hypertonic cytosol' or 34 times greater than the 'isotonic cytosol' (see table 1). Thus, a considerable purification of the enzyme is achieved by using nuclei as a source. Indeed, poly(A) polymerase can be easily purified to homogeneity (as determined by acrylamide gel electrophoresis) from isolated nuclei by a series of ion exchange chromatographies [12], whereas the same scheme of purification applied to a cytosol fraction results in a preparation which is only 40% pure (Rose and Jacob, unpublished observations). At this point, we have not detected any differences in characteristics of the 'free' nuclear and cytosol enzymes.

It should be pointed out that although poly(A) polymerase decreased almost to zero in 'isotonic nuclei', the protein content of this fraction was essentially unaltered. Thus, in all probability only a small percentage of the total nuclear proteins are 'free' to leak out into the cytosol and that poly(A) polymerase is one of these proteins. Indeed, large scale studies indicate that nuclear poly(A) polymerase accounts for 0.01 mg/g liver [12] and thus a complete loss of enzyme protein would not be detected in the present investigation. An equilibrium may exist between nuclear and cytosol enzymes which could allow for a rapid buildup of poly(A) polymerase in the nucleus in response to physiological stimuli which call for increased polyadenylation. Although the levels of poly(A) polymerase present in 'hypertonic nuclei' appear to respond to various physiological parameters [12,16,17], it is not yet known whether the amount of enzyme detected in the 'hypertonic cytosol' is altered in a similar manner to these parameters or whether the two pools of enzyme are regulated independently. Such studies are now in progress.

* Nuclear sap refers to chromatin-free nucleoplasm; see [4].

** Enzyme activity still bound after several isotonic washings

## Acknowledgements

The authors wish to thank Ms Patricia Ruch for technical assistance. This work was supported by USPHS Grant CA-15733 from the National Cancer Institute.

## References

[1] Edmonds, M. and Abrams, R. (1960) J. Biol. Chem. 235, 1142–1149.
[2] Hyatt, E. A. (1967) Biochim. Biophys. Acta 142, 246–253.
[3] Niessing, J. and Sekeris, C. E. (1973) Nature New Biol. 243, 9–12.
[4] Jacob, S. T., Roe, F. J. and Rose, K. M. (1976) Biochem. J. 153, 733–735.
[5] Jacob, S. T. and Schindler, D. G. (1972) Biochem. Biophys. Res. Comm. 48, 126–134.
[6] Jacob, S. T., Morris, H. P. and Schindler, D. G. (1972) Science 178, 639–640.
[7] Jacob, S. T., Rose, K. M. and Morris, H. P. (1974) Biochim. Biophys. Acta 361, 312–320.
[8] Wilkie, N. M. and Smellie, R. M. S. (1968) Biochem. J. 109, 485–494.
[9] Rose, K. M. and Jacob, S. T. (1975) J. Cell. Biol. 67, 739.
[10] Klemperer, H. G. (1963) Biochim. Biophys. Acta 72, 416–420.
[11] Tsiapalis, C. M., Dorson, J. W., DeSante, D. M. and Bollum, F. J. (1973) Biochem. Biophys. Res. Comm. 50, 737–743.
[12] Rose, K. M. and Jacob, S. T. (1976) Eur. J. Biochem., in press.
[13] Rose, K. M., Morris, H. P. and Jacob, S. T. (1975) Biochem. 14, 1025–1032.

[14] Winters, M. A. and Edmonds, M. (1973) J. Biol. Chem. 248, 4756–4762.
[15] Tsiapalis, C. M., Dorson, J. W. and Bollum, F. J. (1975) J. Biol. Chem. 250, 4486–4496.
[16] Jacob, S. T., Rose, K. M. and Munro, H. N. (1976) Biochem. J., in press.
[17] Jacob, S. T., Jänne, O. and Rose, K. M. (1975) in: Regulation of Growth and Differentiated Function in Eukaryote Cells (Talwar, G. P., ed.), pp. 369–378, Raven Press, New York.
[18] Chauveau, J., Moulé, Y. and Rouiller, C. (1956) Exptl. Cell Res. 11, 317–321.
[19] Jacob, S. T., Steele, W. J. and Busch, H. (1967) Cancer Res. 27, 52–60.
[20] Rose, K. M., Ruch, P. A., Morris, H. P. and Jacob, S. T. (1976) Biochim. Biophys. Acta 432, 60–72.
[21] Rose, K. M. and Jacob, S. T. (1974) Biochem. Biophys. Res. Comm. 60, 390–397.
[22] Wilt, F. H. (1973) Proc. Natl. Acad. Sci. USA 70, 2345–2349.
[23] Diez, J. and Brawerman, G. (1974) Proc. Natl. Acad. Sci. USA 71, 4091–4095.
[24] Bennett, T. P. (1967) Nature 213, 1131–1132.