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AFFINITY CHROMATOGRAPHY OF POLY(A) POLYMERASE ON ATP–SEPHAROSE

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1. Introduction

In the cell nucleus, poly(A) polymerase (EC 2.7.7.19) very probably catalyzes the non-transcriptive addition of poly(A) to hnRNA-molecules assumed to be the precursors to cytoplasmic mRNA [1,2]. Poly(A) polymerase from various tissues and cells has been purified by conventional procedures of protein purification [2]. Three distinct forms of poly(A) polymerase from rat liver nuclei have been resolved by chromatography on CM-cellulose [3]. In these experiments a 200-fold purification has been achieved.

Here we wish to describe a purification procedure which is based on the affinity of poly(A) polymerase to its own substrate, ATP. Poly(A) polymerase can be bound specifically and reversibly to immobilized ATP. Affinity chromatography of poly(A) polymerase yielded a 1560-fold purification at a recovery of 57%. Moreover, an enzyme system from rat liver nuclei catalyzing the non-transcriptive and primer-RNA-dependent synthesis of poly(U), poly(G) and poly(C) could be adsorbed to ATP–Sepharose and dissociated with ATP.

2. Methods

ATP–Sepharose was prepared according to Lamed and Oplatka [4]. Poly(A) polymerase and ribohomopolymer polymerase (poly(U)-, poly(G)- and poly(C)-polymerase) were extracted from rat liver nuclei and chromatographed on Bio-Gel A 1.5 m and DEAE–Sephadex as previously described [3,5]. Enzyme activity was measured in a reaction mixture containing in 0.2 ml: 20 mM Tris–HCl, pH 8.0, 1 mM creatine phosphate, 5 μg creatine phosphokinase, 0.1 mM of either ^{3}H-labelled ATP, UTP, GTP or CTP (2–4 μCi), 0.2 A_{260} units of nuclear RNA, 5 mM 2-mercaptoethanol and variable amounts of enzyme protein. Incubations were at 37°C for 2 h. The radioactivity incorporated was measured as described [3]. Protein was determined according to Lowry et al.

3. Results and discussion

ATP–Sepharose was prepared by suspending 3 g of Sepharose 4B adipic acid dihydrazide in sodium acetate buffer containing 5–7 μmol oxidized ATP. The use of non-charged spacers like hydrazides in affinity chromatography should prevent ionic interactions between proteins and the agarose derivative although hydrophobic interactions are likely to occur [6]. Therefore, ATP–Sepharose was washed extensively with purified [7] bovine serum albumin, a protein known to bind to hydrophobic chains [8]. If this step was omitted poly(A) polymerase was irreversibly bound to ATP–Sepharose.

In a typical experiment (fig. 1) 16.2 mg protein in buffer A (10 mM Tris–HCl, pH 7.9, 2mM $MgCl_2$, 2 mM 2-mercaptoethanol, 0.25 mM EDTA, 50 mM NH_4Cl and 20% glycerol) was applied to an ATP–Sepharose column previously washed with buffer A containing 2 mg bovine serum albumin/ml, followed by a wash with buffer A lacking serum albumin. Although almost all the protein passed through the gel unretarded, no poly(A) polymerase activity was found in the flow-through fraction or during the washing step of the column. Poly(A) polymerase was desorbed from ATP Sepharose with an ATP-gradient (0–3 mM) as a sharp peak at 0.9 mM ATP. Negligible amounts of enzyme activity were obtained by further elution with NH_4Cl at 0.4 M. The eluted poly(A) polymerase fractions

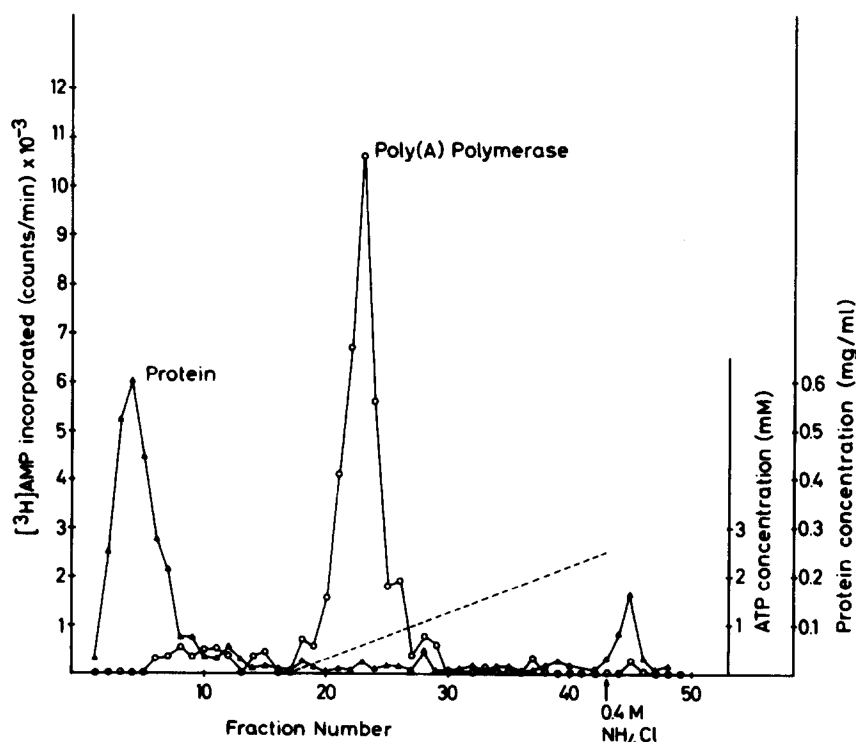

Fig.1. Affinity chromatography of poly(A) polymerase on ATP–Sepharose. After chromatography of nuclear extracts on Bio-Gel and DEAE–Sephadex [5] the enzyme sample was adsorbed to a 1.3 × 8 cm ATP–Sepharose column. The column was washed with 20 ml of buffer A and poly(A) polymerase was eluted with a 0–3 mM ATP-gradient in buffer A. Fractions of 2.5 ml were collected at a flow-rate of 21 ml/h. Since the high level of ATP in the eluate would interfere with enzyme assay, a small DEAE–Sephadex column was closely connected to the ATP–Sepharose column. Alternatively the eluate fractions were extensively dialyzed prior to the enzyme assay.

contained almost undetectable amounts of protein in the range of 4–6 μg/ml which is byond the limits of accurate protein determination. Therefore the extent of enzyme purification may be higher than indicated in table 1. In a control experiment (results not shown) ATP was added to the poly(A) polymerase sample to a final concentration of 1 mM and then passed through ATP–Sepharose. In this case no poly(A) polymerase activity was bound to the adsorbent. This indicates that during affinity chromatography on ATP–Sepharose poly(A) polymerase specifically interacts with the immobilized ATP.

Table 1
Purification of poly(A) polymerase

	Total protein (mg)	Specific activity (nmol/mg protein)	Purification (-fold)	Recovery (%)
Crude Extract	102.8	0.23	1.0	–
Bio-Gel	64.0	2.48	10.8	100.0
DEAE–Sephadex	16.2	7.82	34.1	82.5
ATP–Sepharose	0.012	358.21	1557.0	57.8

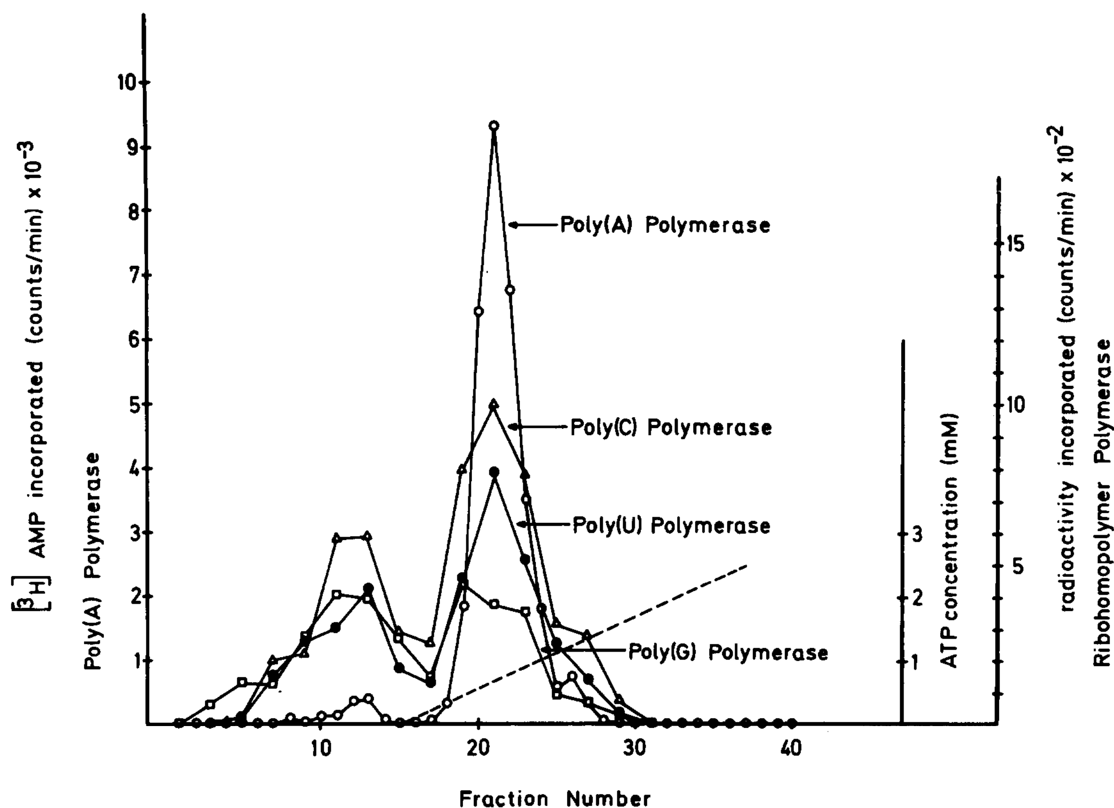

Fig.2. Affinity chromatography of poly(A) polymerase and ribohomopolymer polymerase on ATP–Sepharose. The conditions of chromatography were the same as described in the legend to fig.1.

Dissociation of the enzyme–ligand complex can be achieved by ATP or by salt. In some experiments poly (A) polymerase was eluted from ATP–Sepharose with a 0.05–0.4 M NH_4Cl-gradient at an elution point of 0.175 M NH_4Cl. Under these conditions the extent of enzyme purification was much lower when compared to ATP-eluted poly(A) polymerase due to the unspecific dissociation of other proteins.

Besides poly(A) polymerase, extracts from rat liver nuclei contain an enzyme system capable of synthesizing RNA-primed poly(U), poly(G) and poly(C) [3,5]. The ribohomopolymer polymerase co-purifies together with poly(A) polymerase on Bio-Gel and DEAE–Sephadex but both enzymes can be separated from each other by chromatography on CM-cellulose [3]. Affinity chromatography of poly(A) polymerase and ribohomopolymer polymerase on ATP–Sepharose (fig. 2) again shows the complete adsorption of poly(A) polymerase. Surprisingly, the majority of ribohomopolymer polymerase activity is also retained by ATP–Sepharose and elutes together with poly(A) polymerase at about 0.9 mM ATP. The binding to ATP–Sepharose and the identical elution point of poly(A) polymerase and ribohomopolymer polymerase reflects a similar affinity of both enzymes to ATP. In fact, earlier results [9] have shown that ATP, although not being a substrate, largely inhibited the synthesis of poly(U), poly(G) and poly(C), probably by binding to the enzyme protein.

Affinity chromatography provides an opportunity to selectively isolate and purify macromolecules according to their biological function. In most cases a substrate analogue inhibitor is used as a selective adsorbent because of its high affinity to the macromolecule, but cofactors, effectors and, in special cases, substrate may also be used [10,11]. Our results show that the affinity of immobilized ATP under our experimental

conditions is strong enough to reversibly adsorb poly(A) polymerase and ribohomopolymer polymerase. For the purification of poly(A) polymerase, affinity chromatography on ATP–Sepharose is clearly superior to conventional chromatographic techniques. The adsorbent with a high binding capacity is easily prepared and affinity chromatography can be performed within a few hours on a small column. The successful application of this method largely depends on the experimental conditions chosen, i.e., mainly to avoid unspecific and irreversible interactions of the enzyme protein to the adsorbent by exhaustively washing the column with serum albumin. Moreover, it has been shown [10] that steric hindrance of the binding between protein and ligand can be minimized by the interposition of a spacer group.

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