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DNA polymerase α-DNA primase from human placenta

Immunoaffinity purification and preliminary characterization

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Highly purified DNA polymerase α-DNA primase from normal human tissue (human placenta) has been prepared by immunoaffinity purification on immobilized anti-human DNA polymerase α monoclonal antibody SJK 287-38. According to data from SDS electrophoresis this preparation consists of subunits of 180, 160, 145, 140 kDa (a cluster of DNA-polymerizing subunits), 73 kDa (function unknown) and 59, 52 kDa (corresponding to primase). Three active enzyme forms of 270, 460 and 575 kDa have been revealed using native electrophoresis followed by detection of DNA polymerase activity.

DNA polymerase α; Immunoaffinity purification; (Human placenta)

1. INTRODUCTION

DNA polymerase α has been suggested to be the main enzyme catalyzing DNA replication in eukaryotes. The purification of DNA polymerases α from mammalian tissues by conventional methods is one of the most difficult problems in preparative biochemistry. The subunit structure, enzymatic features and physicochemical characteristics of these enzymes are very much dependent on the scheme of the preparation procedure [1]. In 1982 a set of monoclonal antibodies were raised against DNA polymerase α from human KB cells [2]. Application of these antibodies to immunoaffinity purification has resulted in the preparation of mammalian enzymes of uniform subunit composition [3–9].

Data on the purification and preliminary characterization of DNA polymerase α-DNA primase from normal human placenta using immobilized monoclonal antibody SJK 287-38 are discussed here.

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2. MATERIALS AND METHODS

DNA from calf thymus, DNA polymerase I (Klenow fragment) and dNTP were from Niktibav (USSR); poly(dT) was from Sigma; [^{3}H]dATP (spec. act. 22×10^3 Ci/mol) was from Izotop (USSR); [α-^{32}P]dTTP (spec. act. 3×10^6 Ci/mol) was from Radiopreparat (USSR). Other reagents were analytical grade. The immunoaffinity column (1 ml) was prepared as in [9].

DNA polymerase activity was measured as in [10]. The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 0.2 mg/ml BSA, 4 mM β-mercaptoethanol, $2A_{260}$/ml activated DNA and 50 μM of each of dATP, dCTP, dGTP and dTTP ([^{3}H]dATP of spec. act. 300 Ci/mol); total volume was 100 μl. One unit of activity was defined as the amount of the protein catalyzing the incorporation of 1 nmol dNMP into acid-insoluble material for 1 h at 37°C.

DNA primase activity was measured in 100 μl of a mixture containing 25 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 0.2 mg/ml BSA, 4 mM β-mercaptoethanol, $0.5A_{267}$/ml poly(dT), 25 μM [^{3}H]dATP (spec. act. 300 Ci/mol), 1.5 mM ATP and 25 U/ml DNA polymerase I (Klenow fragment). One unit of primase activity was defined as the amount of the protein necessary for incorporation of 1 nmol dAMP into acid-insoluble material for 1 h at 37°C.

Protein concentration was determined according to [11].

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Immediately following normal full-term delivery, the placenta was cut into pieces, frozen by using liquid N_2 and stored at $-50°C$ for no more than 7 days. DNA polymerase α was purified from 1000 g tissue. The enzyme was extracted and chromatographed on phosphocellulose P11 according to [10]. Active fractions were pooled and mixed with 1.2 vols of a saturated solution of ammonium sulfate adjusted to pH 7.0 with ammonia. The protein precipitate was processed as in [9]. Finally, the enzyme was eluted with 0.1 M potassium phosphate (pH 11.5), 1 M KCl and 10% (v/v) glycerol. Protein elution was registered on a Millichrom chromatograph (USSR). Immediately after elution the solution was neutralized to pH 7–8 by addition of KH_2PO_4 and dialyzed vs 50 mM Tris-HCl (pH 7.5), 4 mM β-mercaptoethanol, 0.5 mM EDTA, 50% (v/v) glycerol and stored at $-20°C$.

3. RESULTS AND DISCUSSION

In previous experiments we faced great difficulties in the conventional purification of DNA polymerase α from human placenta [10]. Here, the immobilized anti-human DNA polymerase α monoclonal antibody SJK 287-38 was used to prepare the enzyme. DNA polymerase α was extracted from placental tissue, enriched by phosphocellulose chromatography and finally purified on the antibody column. Alkaline elution of the bound DNA polymerase was most effective as was the case with the enzyme from calf thymus [9]. The optimal pH of the elution buffer containing 0.1 M potassium phosphate, 1 M KCl and 10% (v/v) glycerol was determined to be about pH 11.3–11.5. Lower pH values did not allow the desorption of catalytic polypeptides from the immunoaffinity column. pH shift to pH 12–13 resulted in complete and irreversible inactivation of the eluted enzyme. The results of purification are presented in table 1. 1 kg processed placenta yielded about 0.11 mg DNA polymerase-primase with a specific activity of 25000–30000 U/mg for the polymerase and 10000–15000 U/mg for the primase.

Table 1

Immunoaffinity purification of DNA polymerase α from human placenta

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)
(1) Crude extract	70000	n.d.	n.d.
(2) Phosphocellulose	410	92000	220
(3) Antibody column	0.11	3000	27000

n.d., not determined

According to the data from SDS-denaturing gel electrophoresis the DNA polymerase-primase preparation was near homogeneous (fig.1). A typical enzyme preparation contained a cluster of catalytic subunits of 180, 160, 145 and 140 kDa, most probably as a result of the differing degrees of proteolysis of commonly translated product [7]. Polypeptides of 73, 59 and 52 kDa were also observed. The function of the 73 kDa polypeptide which is typically present in DNA polymerase α preparations is still unknown. The 52 and 59 kDa polypeptides are responsible for DNA primase activity [13].

The polymerase-primase preparation was further analysed by means of gradient gel electrophoresis under native conditions with the subsequent detection of DNA polymerase activity by overlay assay [14]. The results are presented in fig.2. Distribution of the protein from the origin to a position corresponding to approx. 270 kDa was observed (fig.2A). This may be due to either aggregation or partial denaturation of the protein. Using the overlay assay of DNA polymerase activity, distinct spots of three catalytically active forms of 270, 460 and 575 kDa were detected (fig.2B).

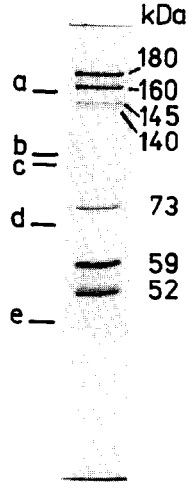

Fig.1. SDS gel electrophoresis of human placenta DNA polymerase α-DNA primase immunoaffinity purified with monoclonal antibody SJK 287-38. 6 μg polymerase-primase was separated on an 8% polyacrylamide gel as described by Laemmli [12]. The gel was stained with Coomassie G-250. Standard protein markers: (a,c) β- and δ-subunits of RNA polymerase from *E. coli*, 155 and 85 kDa; (b) phosphorylase *b*, 93 kDa; (d) BSA, 67 kDa; (e) ovalbumin, 43 kDa.

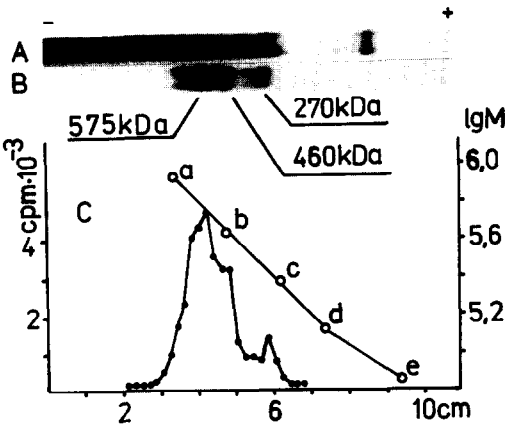

Fig.2. 3–20% gradient gel electrophoresis of immunopurified DNA polymerase α-DNA primase from human placenta under native conditions. Separation of 1.5 μg protein and determination of DNA polymerase activity were carried out according to [14] slightly modified as in [10]. (A) Silver staining of the protein according to [15]; (B) autoradiography of DNA polymerase activity; (C) scintillation counting of agarose gel cut into 2-mm strips (●—●); molecular mass calibration (○—○). Markers: (a) ferritin dimer, 900 kDa; (b) ferritin, 450 kDa; (c) catalase, 240 kDa; (d) BSA dimer, 134 kDa; (e) BSA, 67 kDa.

The relative activities of these forms were determined by scintillation counting of overlay assay agarose gel, cut into 2-mm strips after autoradiography (fig.2C). A band of protein of apparent molecular mass 90 kDa (fig.2A) showed no DNA polymerase activity and might correspond to a free primase. The above data are insufficient for definite conclusions as to the nature of the given active forms of DNA polymerase α. Attempts are currently being made in order to determine the composition and functional characteristics of these forms.

Immunoaffinity chromatography has therefore been rather successfully employed for the isolation of highly purified DNA polymerase α from human placenta. The subunit structure of this preparation is similar to that of other eukaryotic α-polymerases [3–9]. The results obtained demonstrate the possibility of formation of various forms of DNA polymerase α that can be considered as homogeneous according to denaturing gel electrophoresis data.

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