plasmid DNA. Those fragments represent the homologous Ti-plasmid DNA segment 8 μm long (15% of the Ti-plasmid).

An argument that this region of the plasmid contains genes essential for the expression of oncogenicity came from the analysis of a deletion mutant in the Ti-plasmid (Hernalsteens et al., 1975). Since this deletion mutant is unable to confer oncogenicity and has lost exactly those two fragments of Ti-plasmid produced by digestion with enzyme EcoRI that are present in all Agrobacterium tumefaciens strains, we have good indications for the correlation between this general Ti-plasmid DNA segment and the ability for tumour induction.


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**Poly(Adenosine Diphosphate Ribose) Polymerase Purification in an Aqueous/Organic-Solvent System**

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The eukaryotic-cell nucleus contains an enzyme that catalyses the sequential transfer of the ADP-ribose moiety of NAD⁺ to nucleoproteins, resulting in the formation of a homopolymer of ADP-ribose residues [poly(ADP-ribose)], linked by 1'-2' glycosidic bonds; nicotinamide is released in the same reaction (Chambon et al., 1963; Fujimura et al., 1967; Nishizuka et al., 1967, 1969; Ueda et al., 1975a; for a review see Hilz & Stone, 1976). Poly(ADP-ribose) polymerase has been identified as the enzyme catalysing this reaction. Approx. 90% of the total enzyme activity is associated with the chromatin (Nishizuka et al., 1969). The physiological role of poly(ADP-ribose) and of the ADP-ribosylation of chromosomal proteins in nuclear function is unclear. The results of several investigations suggest that poly(ADP-ribose) may be involved in DNA replication and cell proliferation (Burzio & Koide, 1970, 1971; Kidwell & Burdette, 1974). Evidence suggesting the existence of poly(ADP-ribose) *in vivo* has also been reported (Stone & Hilz, 1975; Hilz & Stone, 1976). Poly(ADP-ribose) polymerase has been purified from rat liver 6000-fold (Okayama et al., 1976), from calf thymus 5500-, 540-130- and 10-fold [Ueda et al. (1975b), Okazaki et al. (1976), Yoshihara (1972), Yamada et al. (1971) respectively], and from pig thymus 2500-fold (Tsopanakis et al., 1976).

We have succeeded in purifying the enzyme from pig thymus nuclei 7500-fold, by using an aqueous/organic-solvent system.

**Preparation**

All steps were conducted at 0–4°C. Frozen pig thymus (50g) was sliced and homogenized in 200ml of 0.25m-sucrose containing 3mM-MgCl₂ in 50% (v/v) ethylene glycol. The homogenate was filtered through six layers of muslin and the filtrate was centrifuged at 1400g for 15min. The pellets were resuspended in 0.25m-sucrose containing 3mM-MgCl₂ in 50% (v/v) ethylene glycol, then centrifuged through 2.2m-sucrose containing 3mM-MgCl₂ at 70000g (rₑ 9.86cm) for 60min. The nuclei were suspended in 0.25m-sucrose containing 3mM-MgCl₂ in 50% (v/v) ethylene glycol and extracted with 0.5m-NaCl in 0.1m-Tris/HCl, pH 8.0, in 50% (v/v) ethylene glycol,
by shaking for 30 min. Centrifugation of the extract at 134,000 g (r_{av}, 5.9 cm) for 90 min separated the 0.5 M-NaCl extract from DNA and histones, although low-molecular-weight DNA remained in solution in the extract. There was a consistently large increase in total enzyme activity in 0.5 M-NaCl extract compared with the nuclei (Table 1). This increase was not apparent at further stages. The explanation for this observation is not apparent, but it may be due to the presence of ethylene glycol (see under 'Discussion'). It may also be due to inhibition of the specific glycohydrolase, the loss of an activator in subsequent steps or protein acceptors may be removed on further purification.

The enzyme was purified by successive column chromatography on Sephacryl S-200, hydroxyapatite, both in the presence of 50% (v/v) ethylene glycol, and then on CM-cellulose CM-52 in aqueous solution. The enzyme activity was assayed as described in Table 1 and increased 7525-fold from nuclei with an overall yield of 70% (Table 1).

**Results**

Table 1 summarizes the procedures that were used for purifying poly(ADP-ribose) polymerase from pig thymus nuclei. Figs. 1(a), 1(b) and 1(c) represent the elution patterns obtained at each step. Fig. 1(a) shows that part of the enzymic activity was eluted in the void volume. This activity presumably represents chromatin-bound enzyme. Low-molecular-weight DNA was present in the 0.5 M-NaCl extract and was eluted in the void volume. This was confirmed by measuring the A_{260} of the column fractions, and by directly measuring the DNA in the enzymically active fractions by a fluorimetric method (Kissane & Robbins, 1958). There was also some DNA in peak I (fraction 18) (0.270 mg/mg of protein), but none in peak II (fraction 28). Both fractions were loaded on hydroxypatite columns. The peak-I elution pattern is illustrated in Fig. 1(b). The peak-II profile showed multiple peaks of enzymic activity, the last of which was eluted at 0.42 M-potassium phosphate buffer, pH 7.5, as was the main enzymically active peak shown in Fig. 1(b). The multiplicity of enzymically active peaks may be due to partial enzyme denaturation, to heterogeneity of the enzyme molecules (isoenzymes) or to association of enzyme molecules with a variety of other chromatin proteins.

Only the material in peak I (Fig. 1a) was used for further purification. This material was separated into two enzymically active fractions on hydroxypatite (Fig. 1b), being eluted around 0.35 M- and 0.42 M-sodium phosphate buffer, pH 7.5. The enzymically active peak (fraction 21), eluted from the hydroxypatite column around 0.42 M-buffer (Fig. 1b), was chromatographed on a CM-cellulose CM-52 column (Fig. 1c). The CM-cellulose column gave more satisfactory results in aqueous solution than in aqueous ethylene glycol. One major single peak of enzymic activity was eluted at around 0.81 M-NaCl.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the material from this peak (30 μg) revealed a single band after staining with Coomassie Brilliant Blue, having an apparent mol.wt. of 63,500.

**Discussion**

The ethylene glycol solvent was tested because we wanted to use low-temperature chromatography (−20°C) (Douzou, 1973) to minimize the enzyme inactivation that takes place during purification, even at near-zero temperatures (4°C). Ethylene glycol was the only organic solvent among those tested in our laboratory (methanol, ethanol, glycerol, diethylformamide) at various concentrations from 10 to 50% (v/v) that did not precipitate or inactivate the enzyme. There was no difference in enzyme activity when assayed in aqueous solution or in 50% (v/v) ethylene glycol. Aqueous ethylene glycol (50%, v/v) freezes at −45°C, does not show deleterious effects on enzyme activity, and its solvation effects are reversed by its removal (Douzou, 1973).

We used the aqueous ethylene glycol system at 4°C to evaluate differences in yield and stability of the enzyme in comparison with the aqueous one previously used (Tsopanakis et al., 1976). It seems that even at 4°C there is an impressive improvement in overall yield and an increase in specific activity compared with our previous results; also, there is a 30-fold purification and a 687% yield increase (Table 1) of the enzyme.
Fig. 1. Chromatography of poly(ADP-ribose) polymerase from pig thymus nuclei

(a) Chromatography on a column (65 cm x 2 cm) of Sephacryl S-200 equilibrated in 10 mM-Tris/HCl, pH 8.0, containing 500 mM-NaCl, 2.5 mM-MgCl₂ and 50% (v/v) ethylene glycol. Elution was with the same buffer. A portion (4 ml) of a 0.5 M-NaCl extract (see Table 1) in 50% (v/v) ethylene glycol was chromatographed at a rate of 13.5 ml/h. The fraction volume was 13.3 ml. (b) Chromatography on a column (7 cm x 2 cm) of hydroxyapatite equilibrated in 1 mM-potassium phosphate buffer, pH 7.3, containing 500 mM-NaCl and 50% (v/v) ethylene glycol. A 1-500 mM-potassium phosphate gradient, pH 7.5, containing 500 mM-NaCl and 50% (v/v) ethylene glycol (total volume 120 ml) was used for elution at a flow rate of 7 ml/h. The
fraction volume was 14ml. (c) Chromatography on a column (8 cm x 2 cm) of CM-cellulose CM-52 equilibrated in 20 mM-potassium phosphate buffer, pH 8.4, containing 500 mM-NaCl. A NaCl gradient of 0.5-1.0 M in the above buffer (total volume 300 ml) was used for elution, at a flow rate of 75 ml/h. The fraction volume was 15 ml. In all three cases samples were taken from each fraction and assayed for enzyme activity and for protein.

○, Enzyme activity (c.p.m.); ●, specific enzyme activity (munits/mg of protein). ———, Phosphate or NaCl concentrations. Note that the scale of specific enzyme activity increases 5 times from (a) to (b), and 10 times from (b) to (c). The enzyme-activity scales are identical for all three graphs.

Fraction 18 from the Sephacryl S-200 separation (4 ml) was used for the hydroxyapatite separation, and fraction 21 of the hydroxyapatite separation (4 ml) was used for the CM-cellulose separation.

Table 1. Purification of poly(ADP-ribose) polymerase

The starting material was 50 g of frozen pig thymus. Enzyme activities are expressed in munits. A unit of enzyme activity is defined as that activity which incorporates 1 μmol of NAD⁺ into acid-insoluble material in 1 min at 25°C. Protein concentration was determined by the method of Lowry et al. (1951). The enzyme activity was measured by the incorporation of radioactivity from [³H]adenosine-labelled NAD⁺ into acid-insoluble material. The incubation mixture contained 91 mM-Tris/HCl, pH 8.4, 9 mM-MgCl₂, 4.5 mM-NaF, 9.1 mM-dithiothreitol, 100 μl of nuclear suspension or enzyme solution and 192.3 pmol of [³H]NAD⁺, in a total volume of 550 μl. In all the enzyme assays, except those using nuclei as enzyme source, 40 μg of DNA and 40 μg of histone were included. Incubation was at 25°C for the appropriate time and the reaction was stopped by adding 1 ml of cold 20% (w/v) trichloroacetic acid containing 4 mg of NAD⁺/ml. In step 3, one-fifth of the total 0.5 M-NaCl extract was used, and in step 4 one-third of fraction 18 was used. The precipitate was collected and washed of GF/C glass-fibre filters and the radioactivity was measured in a liquid-scintillation spectrometer.

<table>
<thead>
<tr>
<th>Step</th>
<th>Fraction no. (see Fig. 1)</th>
<th>Total protein (mg)</th>
<th>Total enzyme activity (munits)</th>
<th>Overall yield of enzyme activity (%)</th>
<th>Specific enzyme activity (munits/mg of protein)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nuclei</td>
<td>-</td>
<td>315</td>
<td>43.68</td>
<td>100</td>
<td>0.138</td>
</tr>
<tr>
<td>2.</td>
<td>0.5 M-NaCl extract</td>
<td>-</td>
<td>72</td>
<td>300.00</td>
<td>687</td>
<td>4.17</td>
</tr>
<tr>
<td>3.</td>
<td>Sephacryl S-200 (peak I)</td>
<td>18</td>
<td>1.5</td>
<td>35.07</td>
<td>80.3</td>
<td>23.38</td>
</tr>
<tr>
<td>4.</td>
<td>Hydroxyapatite</td>
<td>21</td>
<td>0.304</td>
<td>38.93</td>
<td>89.1</td>
<td>128.1</td>
</tr>
<tr>
<td>5.</td>
<td>CM-cellulose CM-52</td>
<td>14, 15</td>
<td>0.030</td>
<td>31.29</td>
<td>71.6</td>
<td>1043.0</td>
</tr>
</tbody>
</table>
during the 0.5 m-NaCl extraction, as compared with a 22-fold purification and a 146% yield of the aqueous method used before (Tsopanakis et al., 1976). This result may be due to the presence of ethylene glycol in the solutions.

Ethylene glycol seems to play a 'protective role' for the enzyme, independent of the temperature effect it may have at -20°C.

It is noteworthy that the most purified fraction still shows apparent enzyme activity in the absence of added protein acceptors. This may be due either to self modification of the enzyme molecules or of the added histone H1, or it may be due to the synthesis of long acid-insoluble free polymers.

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Study of the Translational Stability of Messenger Ribonucleic Acids for
Lytechinus pictus and HeLa-Cell Histones Injected into Xenopus Oocytes

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We have previously shown that the removal of the poly(A) segment from globin mRNA markedly decreases its stability when injected into frog oocytes (Nudel et al., 1976). To strengthen further the idea that the poly(A) is required to ensure the stability of eukaryotic mRNA, we decided to study the stability of an mRNA in oocytes which naturally lacks poly(A). Histone mRNA was chosen (Adesnik & Darnell, 1972).

RNA fractions enriched in histone mRNA were prepared from either early embryos of Lytechinus pictus (Weinberg et al., 1972) or synchronized HeLa cells in S-phase (Gallwitz & Breindl, 1972). Oocytes (100) from a single Xenopus laevis female were injected with 50 nl each of aqueous

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