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Two subforms of eukaryotic topoisomerase I

Purification and structure–function relationships

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A new method for isolation of eukaryotic topoisomerase I from calf thymus and from Jurkat-1 cells using HPLC has been developed. The method allows quantitative purification of high molecular weight topo I and of two low molecular weight fractions differing by their isoelectric points. It has been suggested that these fractions be characterized as two subforms of the enzyme possessing structural and functional differences. The differences in their specific activities, sensitivity to camptothecin and in their proteolytic digestion maps have been demonstrated for the two enzymes.

Topoisomerase I; Subform; HPLC; Hydrophobic interaction chromatography; Camptothecin resistance

1. INTRODUCTION

The type I topoisomerases (topo I) are key enzymes in the control of DNA topological state changes necessary for many genetic processes [1].

The procedure for isolation of topo I from calf thymus [2] and some other tissues [3] yields several polypeptides of different molecular weight. The same phenomenon was found in prokaryotic cells [4]. This might be due to partial proteolysis of the native topo I of high molecular weight. Here we suggest that this phenomenon can be caused also by another reason.

2. EXPERIMENTAL

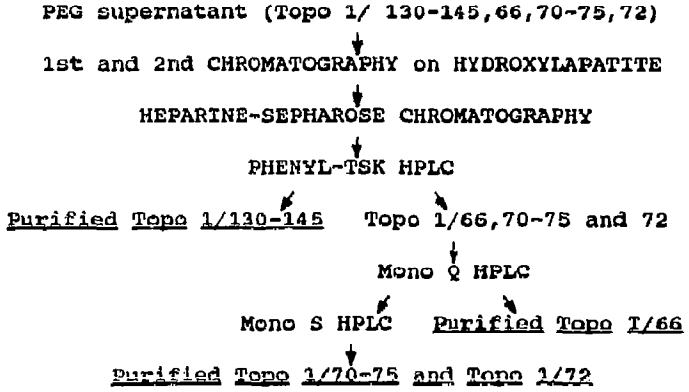
2.1. *Purification of type I topoisomerase*

Topo I was initially purified from calf thymus and Jurkat-1 cells as described [2]. Then we developed our original HPLC protocol. The Altex (Beckman, Model 332) HPLC system was used. All steps were carried out at 4°C with buffers containing 1.4 mM 2-mercaptoethanol (ME) and 1 mM PMSF; the flow rate was 0.5 ml/min.

The active fraction eluting from a heparin–Sepharose column was diluted to a concentration of 5% glycerol. Solid ammonium sulfate (AS) was slowly added to 33% saturation, and after stirring for 60 min the precipitate was removed by centrifugation (13,000 × *g*, 15 min). The supernatant was applied to Phenyl 5PW (TSK column), equilibrated with buffer A, containing 50 mM potassium phosphate buffer (KPB), pH 7.2, and 33% saturation with AS. Elution was performed with an increasing linear gradient of buffer B, containing 50 mM KPB, pH 7.2, and 30% ethylene glycol. After dialysis against 50 mM KPB, pH 7.2, and 20% glycerol (buffer C), specific topo I activity was found in the bound and unbound fractions. The last was loaded onto a Mono Q column, and the unbound fraction from the Mono Q column was then loaded directly onto a Mono S column: at this point all the activity was bound to the column. Both columns were equilibrated with buffer C and in both cases the elution was performed with an increasing linear gradient of 50 mM KPB, pH 7.2, 20% glycerol and 1 M NaCl (buffer D).

2.2. *Other procedures*

Topo I activity was measured by the relaxation of supercoiled plasmid DNA (pUC19) [5]. One unit was defined as the amount of enzyme that relaxes half of the added plasmid DNA (1 μg) in 10 min at 25°C. The assay was also carried out with camptothecin (CPT) at different concentrations. Isoelectric focusing with subsequent immunodetection was done as in [6]. Partial digestion of subforms of topo I with protease from the V8 strain of *Staphylococcus aureus* (Boehringer-



Scheme 1. Scheme of purification of topoisomerase I.

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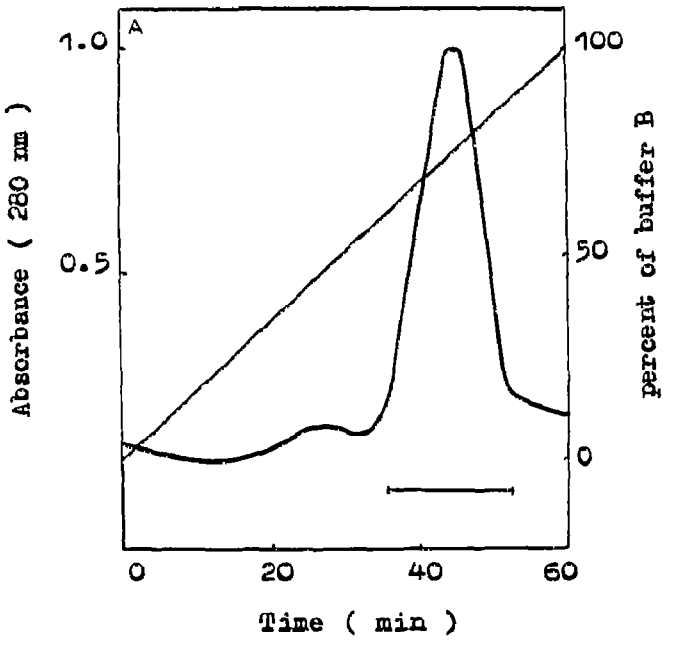
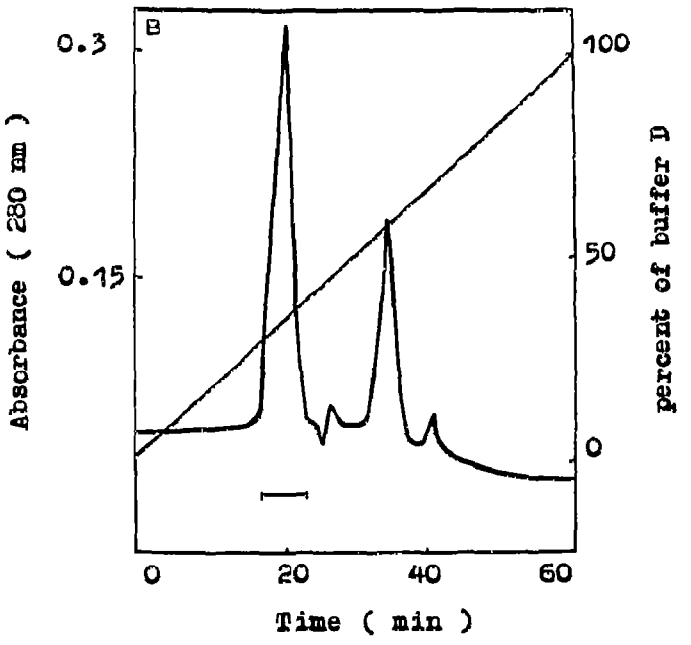
Abbreviations: ME, 2-mercaptoethanol; PMSF, phenylmethylsulfonylfluoride; AS, ammonium sulfate; KPB, potassium phosphate buffer; CPT, camptothecin; topo I/130–145, 66, 70–75, 72, topo I of 130–145, 66, 70–75, 72 kDa, respectively; HPLC, high performance liquid chromatography.





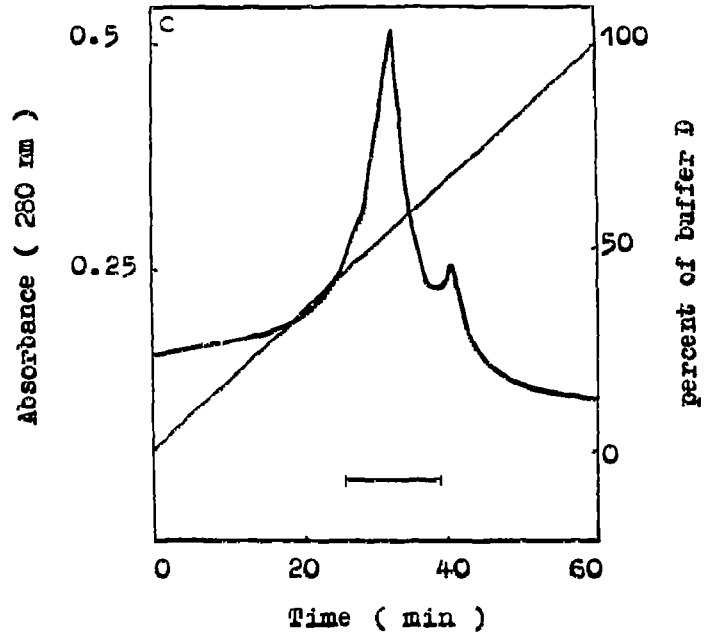


Fig. 1. HPLC of topo I on Phenyl 5PW (A), Mono Q (B) and Mono S (C) columns. The active fractions are indicated between the bars.

Mannheim) was carried out in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% glycerol and 150 mM NaCl. A protease to topo I ratio of 1:50 was used in all cases. SDS-PAGE was done as described [7].

3. RESULTS AND DISCUSSION

The proposed purification procedure is displayed in Scheme 1. The introduction of the hydrophobic interaction HPLC step allowed us to separate low and high molecular weight topo I and gave a quantitative yield of topo I/130–145 (Fig. 1A). Mono Q HPLC was the

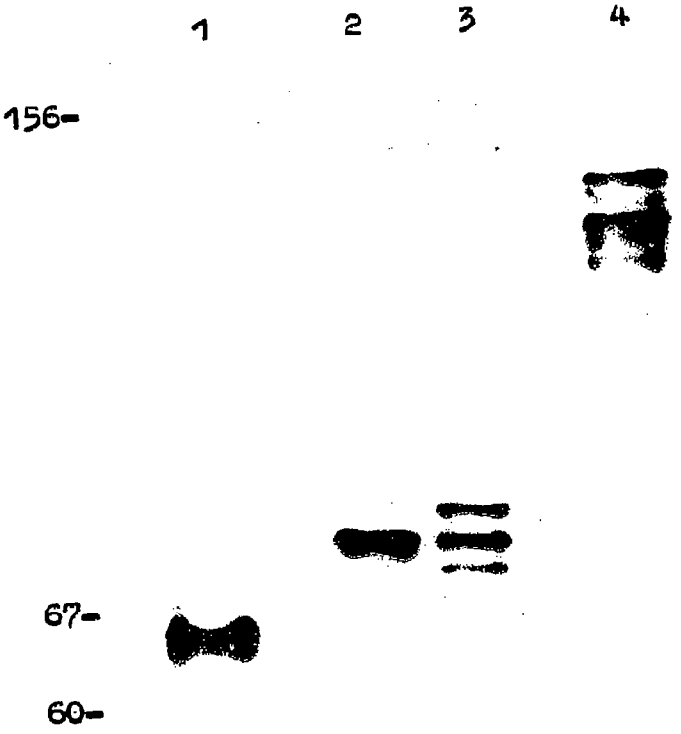


Fig. 2. SDS-PAGE (7.5% gel) of the purified subforms of topo I. Lane 1, the Mono Q fraction. The two separated fractions of topo I were purified on a Mono S column: lane 2, the last 1/3 of the protein peak (topo I/72); lane 3, its first 2/3 (topo I/70–75); lane 4, topo I eluted from Phenyl 5PW. Immunoblotting (data not shown) indicated that all these proteins were topo I.

Table I

Purification of topo I subforms from calf thymus (500 g tissue)

Fraction	Total volume (ml)	Total protein (mg)	Specific activity (U/mg)	Yield (%)
I. PEG supernatant	1.000	2.000	175	100
II. 1st hydroxylapatite	300	150	2.0×10^3	86
III. 2nd hydroxylapatite	50	10	2.6×10^4	74
IV. Heparin–Sepharose	7	4.1	6.2×10^4	72
V. HPLC on Phenyl 5PW	2.5	2.5	5.0×10^4	36
VI. HPLC on Mono Q	1.5	0.4	1.5×10^5	17
VII. HPLC on Mono S	1.5	0.75	7.0×10^4	15

The total yield of all topo I fractions (steps V–VII) was 68%. The concentration of proteins was estimated as in [8].

second step in our protocol (Fig. 1B). Activity bound to the column proved to be homogeneous topo I/66. A pI = 6.0 was determined for this topo I fraction, whereas the usual pI of topo I is in a narrow range between 8.1 and 8.3. The large loss of specific activity observed by Schmitt et al. at the last step of purification of topo I on Bio-Rex 70 [2] could account for failure to identify this fraction. The unbound fraction from the Mono Q HPLC step was purified on a Mono S column (Fig. 1C) and gave topo I similar to those from Bio-Rex 70 elution (specifically topo I/72 with pI = 8.1). Each step of the purification procedure is characterized in Table I. SDS-PAGE of all obtained topo I fractions is presented in Fig. 2.

The dramatically different behaviour of these proteins of close molecular weights when using different ion-exchange supports allowed us to suppose that this could be due to structural differences of the topo I fractions. This assumption needed strong functional and structural support.

We showed that the isolated subforms had different proteolytic digestion patterns (Fig. 3), specific activities (2×10^5 and 1×10^5 U for topo I/66 and topo I/72, respectively) and differed greatly in their sensitivity to CPT (Fig. 4). Topo I/72 was inhibited by CPT at 5 μM, whilst topo I/66 appeared to be CPT resistant, even at 625 μM.

The differences in properties of the subforms may be caused by post-translational modification of topo I/66. The observed correlation of the specific activity of topo I with its modification [9] supports this idea, although more probably it can be explained by the structural differences of this subform of topo I. The different sen-

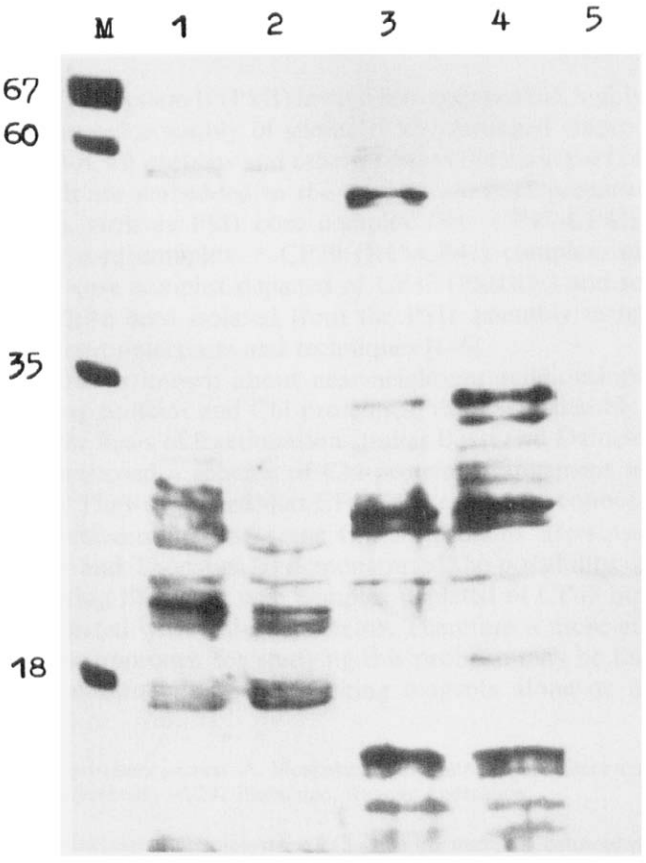


Fig. 3. SDS-PAGE (12.5% gel) of the products of partial digestion of the subforms of topo I with protease from the V8 strain of *Staphylococcus aureus*. Lanes 1 and 2, digestion of topo I/66 with the protease for 16 h at 25°C and for 3 h at 37°C, respectively. Lanes 3 and 4, digestion of topo I/72 with the protease for 16 h at 25°C and for 3 h at 37°C, respectively. Lane 5, V8 protease; lane M, mol. wt. markers in kDa.

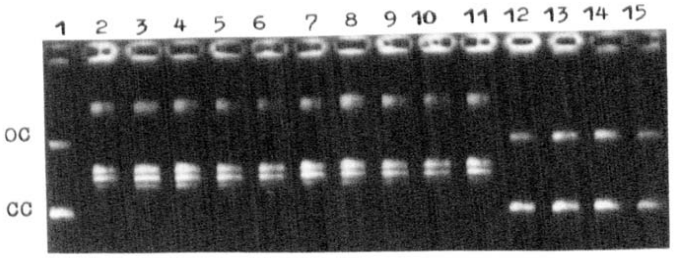


Fig. 4. Effect of CPT on topo I/66 and topo I/72. Lane 1, plasmid DNA pUC19; OC, open circular DNA; CC, closed circular plasmid DNA. The enzymatic activity was assayed in the absence (lanes 2,9) and presence of CPT at 0.2 (lanes 3,10), 1.0 (lanes 4,11), 5.0 (lanes 5,12), 25 (lanes 6,13), 125 (lanes 7,14) and 625 μM (lanes 8,15), respectively.

sitivity to CPT may confirm this proposal as it has been demonstrated that mutations could affect this property of the enzyme [10], although the CPT-resistant topo I described here had no detectable differences in its peptide map in comparison with wild-type topo I. In our investigation two forms of eukaryotic topo I of different sensitivity to CPT had dramatic differences in proteolytic patterns, allowing us to suggest differences of these enzymes in their primary structures.

REFERENCES

[1] Gellert, M. (1981) Annu. Rev. Biochem. 47, 449–479.
[2] Schmitt, B., Buhre, U. and Vosberg, H. (1984) Eur. J. Biochem. 144, 127–134.
[3] Tricoli, J.V. and Kowalski, D. (1983) Biochemistry 22, 2025–2031.
[4] Burrington, M.C. and Morgan, A.R. (1976) Can. J. Biochem. 54, 301–306.
[5] Prell, B. and Vosberg, H.-P. (1980) Eur. J. Biochem. 108, 389–398.
[6] Johnson, T.K., Xuen, K.C.L., Denell, R.E. and Consigli, R.A. (1983) Anal. Biochem. 133, 126–131.
[7] Laemmli, U.K. (1970) Nature 227, 680–685.
[8] Bradford, M.M. (1976) Anal. Biochem. 72, 248–255.
[9] Pommier, Y., Kerrigan, D., Hartman, K.D. and Glaser, R.I. (1990) J. Biol. Chem. 256, 9418–9422.
[10] Andoh, T., Ishii, K., Suzuki, Y., Ikegami, Y., Kusunoki, Y., Takemoto, Y. and Okada, K. (1987) Proc. Natl. Acad. Sci. USA 84, 5565–5569.