Vol. 198, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1994 Pages 387-392

EVIDENCE FOR THE PRESENCE OF TOPOISOMERASE LIKE ACTIVITY IN MITOCHONDRIA OF SACCHAROMYCES CEREVISIAE

Vanishree Murthy and K. Pasupathy*

Radiation Biology and Biochemistry Division
Trombay, Bombay 400 085, India

Received December 1, 1993

Summary: During studies on the enzymology of DNA replication in mitochondria of Saccharomyces cerevisiae, a topoisomerase like activity was detected for the first time. Crude extracts of mitoplast were found to show enzyme activities which could both catenate and relax supercoiled plasmid DNA. Chromatography of the mitoplast lysate on a phosphocellulose column, using Tris-HCl (pH 7.5) buffer containing 0.6 M NaCl as eluent, was found to yield a topoisomerase like activity capable of relaxing supercoiled plasmid DNA (Fraction 1). This fraction was not dependent on ATP for its activity. The other fraction eluting at 1M NaCl showed predominantly catenating activity, which was found to be ATP dependent.

Topoisomerases are ubiquitous enzymes which catalyse the interconversion of topological isomers of a DNA molecule by causing a concerted breakage and religating of the DNA backbone (1). They have an important role in functions relating to topological changes of DNA both during replication, transcription and recombination. They can relax, catenate and decatenate DNA molecules. They are classified into 2 types on the basis of ATP requirement and mechanism of strand breakage (either double or single) and linking number change (2-5).

In eukaryotes the information on topoisomerases is mostly restricted to nuclear enzymes and recently this enzyme has been reported and characterised from mammalian mitochondria (6). We present here the evidence for topoisomerase like activity in yeast mitochondria.

MATERIALS AND METHODS

Strain and growth

Saccharomyces cerevisiae D 27310B haploid strain was used in these studies. Cells were grown on YEPD medium aerobically to log phase.

*Corresponding author.

0006-291X/94 $5.00

Preparation of purified mitochondria

Crude mitochondria were prepared from log phase cells of S. cerevisiae using previously published procedure (7). Crude mitochondria were then purified by centrifugation at 25000 rpm on a discontinuous sucrose density gradient for two hrs using an SW 28 rotor (8).

Assay of topoisomerase

The standard assay for topoisomerase activity was carried out according to Castora and Kelly (9). Reaction mixture (20 µl) contained 25 mM Tris HCl pH 8, 40 mM KCl, 1 mM EDTA, 0.5 mM DTT, 30 µg/ml BSA, 0.5 µg pUC 19 plasmid DNA and different amounts of enzyme preparation and was incubated at 30°C for 30 min. The reaction was stopped by the addition of 2.5 µl of 1% SDS, 50% glycerol and 0.1% bromophenol blue. The reaction mixture was then loaded on a 0.7% agarose gel and electrophoresed for 3 hrs at 80 volts in Tris borate EDTA buffer. The gel was stained with 0.5 µg/ml ethidium bromide and the DNA bands were visualised by illumination with UV lamp.

Gel electrophoresis

Purified enzyme was subjected to electrophoresis on 7.5% polyacrylamide gel. SDS polyacrylamide electrophoresis (10%) was carried according to Laemmli (10).

Estimation of DNA and protein

DNA was estimated using Laberca's method (11) and protein was estimated by the method of Bradford (12).

RESULTS

Mitochondrial DNA topoisomerases have been purified from mammalian sources. We have earlier reported and characterised DNA primase activity from yeast mitochondria (7). In our studies on a replisome complex (unpublished results), it was found that mitochondrial crude extracts showed, apart from other DNA replicating enzymes, the presence of a topoisomerase like activity. Studies were therefore directed towards ascertaining the presence of this enzyme in yeast mitochondria and attempts were made to purify it.

The pure mitochondrial layer obtained from discontinuous sucrose density gradient centrifugation was suspended in 20% sucrose buffer and treated with digitonin to strip off the mitochondrial outer membrane. Any chance contamination of nuclear debris is ruled out by this treatment. The mitoplast was then solubilized in 1 M NaCl in buffer A (50 mM Tris-HCl buffer pH 7.0, 1 mM DTT, PMSF, 10% glycerol) by homogenisation followed by centrifugation at 10000 g for 30 min. The supernatant was dialysed against low salt buffer (0.1 M NaCl). This crude extract was then assayed for topoisomerase activity.

As seen in Fig 1, crude extract was capable of relaxing and catenating supercoiled plasmid DNA. [Both PUC 19 and pBR 322 DNA were used as template and the extract could catenate and relax both the substrate DNAs]. Increasing amounts of crude mitochondrial extract resulted in increased catenation and complete removal of supercoiled DNA.

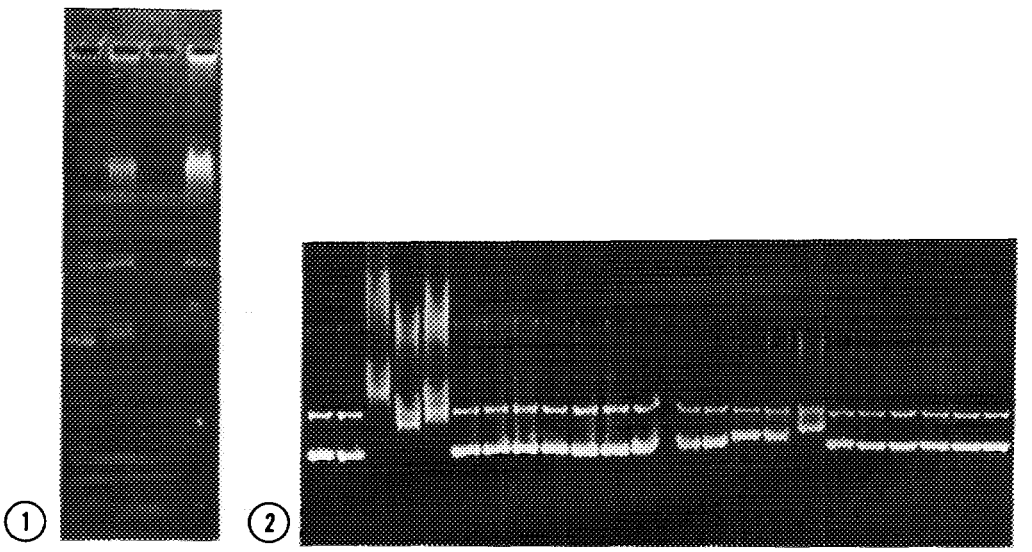

Fig. 1. Detection of topoisomerase activity in crude mitochondrial extract. Logarithmically grown cells of S. cerevisiae were harvested, lysed and mitochondria were isolated. Topoisomerase activity was assayed as described in Materials and Methods using supercoiled plasmide puc19 DNA as the substrate.
Lane 1 plasmid DNA; Lane 2 with 10 µl of crude mitochondrial lysate; Lane 3 with 20 µl of crude mitochondrial lysate.

Fig. 2. Purification of topoisomerase (Fraction 1) on phosphocellular column. Mitochondria were isolated from logarithmically grown yeast cells, lysed and dialysed and loaded on phosphocellular column. The column was eluted with 0.4 and 0.6 M NaCl in buffer A and fractions were assayed for topoisomerase activity.
Lane 1 puc19 DNA; Lane 2 with column wash; Lane 3 with crude mitochondrial lysate; Lanes 4 and 5 with mitochondrial dialysate; Lanes 6-11 with 0.4 M NaCl fractions; Lane 12 onwards with 0.6 M NaCl fraction.

The crude extract (which contained other DNA replicative enzymes) also probably had nuclease activity as seen by the smearing pattern. Topoisomerase was selectively purified from this mitochondrial lysate by fractionating it on a phosphocellulose column (10 x 1 cm) equilibrated with buffer A containing 0.1 M NaCl. After extensive washing with 0.2 M NaCl in buffer A, bound proteins were eluted successively by 0.4 M NaCl, 0.6 M NaCl and 1 M NaCl in buffer A. Fractions of 0.7 ml were collected and assayed for topoisomerase activity. As can be seen in Fig 2 topoisomerase activity was detected in early 0.6 M NaCl fractions (Fraction 1). The enzyme showed a predominant relaxation of plasmid DNA. Active enzyme fractions were pooled and concentrated using a centricon. Further elution of the column with 1 M NaCl yielded a fraction which showed a predominantly catenation activity (Fraction 2)(Fig 3). It is known that Topoisomerase Type 1 is not stimulated by ATP and Type 2 requires ATP for its activity (2-5). As seen in Fig 4, Fraction 1 shows

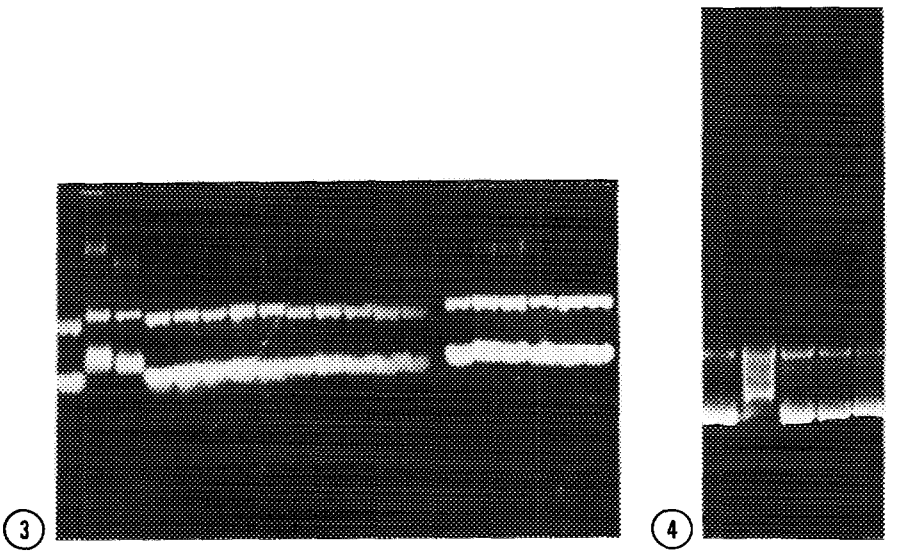

Fig. 3. Purification of Topoisomerase (Fraction 2) (continued). Phosphocellulose column loaded with mitoplast dialysate was further eluted with 0.8 M NaCl and 1 M NaCl in buffer A and topoisomerase was assayed in the fractions.
Lane 1 puc19 DNA; Lane 2 with crude mitochondria lysate; Lane 3 with mitoplast dialysate; Lanes 4-11 with 0.8 M NaCl fractions; Lane 12 onwards with 1 M NaCl fractions.

Fig. 4. Effect of ATP on the activity of Fraction 1 enzyme. Fraction eluting at 0.6 M NaCl were pooled, concentrated and assayed with and without ATP.
Lane 1 puc19 DNA; Lane 2 with crude lysate + 0.5 mM ATP; Lane 3 with Fraction 1 + 0.5 mM ATP; Lane 4 with Fraction 1 + 1 mM ATP; Lane 5 with Fraction 1 + 5 mM ATP.

unaltered activity even in the presence of increasing concentrations of ATP. Thus Fraction 1 seems to resemble Type 1 class of topoisomerases.

The enzyme protein was concentrated and then subjected to electrophoresis on 7.5% PAGE to check the homogeneity. SDS polyacrylamide gel electrophoresis showed 2 major bands of 85 and 90 kDa (Fig 5).

It is known that topoisomerase Type II requires ATP for its activity (2-5). Inclusion of ATP at different concentrations increased activity of Fraction 2 (Fig 6). Efforts are on to purify this fraction. Based on the present results it can be stated that Fraction 1 resembles Type 1 group of topoisomerases in that it has predominantly relaxing activity and is independent of ATP (2) and Fraction 2 which showed catenating ability and is dependent on ATP may resemble Type 2 group of topoisomerases.

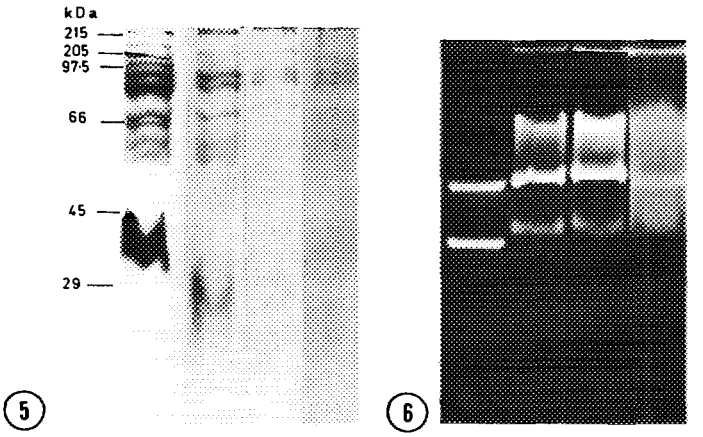

Fig. 5. SDS PAGE profile of Fraction 1. Fraction 1 was analysed in SDS PAGE and stained with Coomassie blue.
Lane 1 crude mitochondrial lysate; Lane 2, 1 M extract of mitoplast; Lane 3, mitoplast dialysate; Lane 4, Fraction 1.

Fig. 6. Effect of ATP on the activity of Fraction 2. Enzyme fractions eluting at 1 M NaCl were pooled, concentrated and assayed with and without ATP.
Lane 1 puc19 DNA; Lane 2 with Fraction 2 + 0.5 mM ATP; Lane 3 with Fraction 2 + 1 mM ATP; Lane 4 with Fraction 2 + 2 mM ATP.

DISCUSSION

These studies have provided evidence to suggest that mitochondria of *S. cerevisiae* may possess enzyme activities that can relax and catenate supercoiled plasmid DNA. Crude extracts showed the presence of both relaxing and catenating activities. Upon purification on phosphocellulose the relaxing activity was predominantly isolated in 0.6 M NaCl eluant. The enzyme tends to show similarity to Type 1 topoisomerases. As is clear from Fig 1 crude extract of mitochondria showed catenating activity also, which was eluted at increasing salt concentration (Fraction 2). This could resemble Type II enzyme which is known to require ATP. Its detection in crude mitochondrial lysate (which is rich in ATP) would mean that endogenous ATP would have sufficed to stimulate its activity.

Topoisomerases play important roles in replicative and repair synthesis of DNA (13). We have reported earlier that mitochondria lack excision repair after UV radiation (14). Recombination repair which is one of the major repair pathways also requires topoisomerases (13). The presence of the enzyme in mitochondria of *S. cerevisiae* thus assumes importance in view of this evidence.

Acknowledgment: The authors wish to thank Dr. K.G. Chetty for helpful discussions.

REFERENCES

1. Gellert, M. (1981) Ann. Rev. Biochem. 50, 879-910.
2. Maxwell, A. and Gellert, M. (1986) Adv. Protein Chem. 38, 69-107.
3. Wang, J.C. (1985) Ann. Rev. Biochem. 54, 665-697.
4. Wang, J.C. (1987) Biochim. Biophys. Acta 909, 1-9.
5. Liu, L.F. (1989) Ann. Rev. Biochem. 58, 351-375.
6. Castora, F.J. and Lazarus, G.M. (1987) Biochemistry 26, 6195-6203.
7. Desai, S.D., Pasupathy, K., Chetty, K.G. and Pradhan, D.S. (1989) Biochem. Biophys. Res. Commun., 160, 525-534.
8. Querol, A. and Barrio, E. (1990) Nuc. Acid. Res. 18, 1657.
9. Castora, F.J. and Kelly, W.G. (1986) Proc. Natl. Acad. Sci. USA 83, 1680-1684.
10. Laemmli, U.K. (1970) Nature 227, 680-685.
11. Laberca, C. and Paigen, K. (1980) Anal. Biochem. 102, 344-352.
12. Bradford, M. (1976) Anal. Biochem. 72, 248-254.
13. Kornberg, A. and Baker, T.A. (1992) in DNA Replication, 379-401.
14. Pasupathy, K. and Pradhan, D.S. (1992) Mut. Res. 273, 281-288.