A BIOLOGICAL ASSAY FOR POLYNUCLEOTIDE LIGASE: RECOVERY OF MARKER ACTIVITY IN DNA-TRANSFORMATION.

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The discovery of an enzyme, polynucleotide ligase, which repairs single strand interruptions in double stranded DNA (Weiss and Richardson, 1967; Becker et al., 1967; Olivera and Lehman, 1967) has furnished an important tool to study the mechanism of DNA repair and recombination. The assay for polynucleotide ligase activity depends on the availability of γ-labeled ATP -32 of high specific activity and polynucleotide kinase, which are required for preparation of the substrate. This requirement may limit the applicability of this enzyme to laboratories familiar with the biochemical techniques required for this assay. We have observed that polynucleotide ligase produced after T4 infection can be reproducibly purified according to the published procedure (Weiss and Richardson) without assay of the individual steps to the point where contaminating nucleases are effectively removed. Purified polynucleotide ligase from phage T4 infected E. coli cells was found to restore marker activity of transforming DNA previously inactivated by "nicking" with pancreatic DNAase. This recovery of marker activity can be used as a simple biological assay for polynucleotide ligase.

MATERIALS AND METHODS

Transformation of the T4 r^+ character by r^+ DNA in E. coli B spheroplasts infected with urea treated rII helper phage has been fully described (F. A. Bautz, 1966). Polynucleotide ligase was obtained from E. coli B cells harvested 2 hr after infection with phage T4 am82 at a multiplicity of 4 and purified according to Weiss and Richardson (1967).

Biological assay of polynucleotide ligase: The DNA substrate was prepared by treating T4 r^+DNA with pancreatic DNAase (8×10^{-3} µg/ml) for 30 min at room temperature; the reaction was stopped by addition of EDTA, followed by dialysis against 0.01 M Tris buffer pH 8.0 containing 0.02 M NaCl. Six µg of nicked DNA were incubated for 40 min at 37°C in a total volume of 0.6 ml containing (in mM): Tris pH 7.6 (14), $MgCl_2$ (3), 2-mercaptoethanol (3), ATP (0.1), and 0.1 µg of protein of phosphocellulose fraction (20 µl direct or diluted with the dilution buffer of Weiss and Richardson (1967)). After incubation 0.2 ml were pipetted into 0.3 ml of boiling 0.01 M phosphate buffer; after boiling for 5 min the tubes were cooled quickly and used directly for the transformation assay.

Chemical assay of polynucleotide ligase: The procedure of Weiss and Richardson (1967) was followed. γ-labeled ATP-32 (spec. act. 540 µc/µmole) was purchased from International Chemical and Nuclear Corp. Polynucleotide kinase was isolated according to Richardson (1965). The P-32 labeled 5'phosphoryl- T4 DNA was separated from the labeled ATP by extensive dialysis instead of the MAK column chromatography described by Weiss and Richardson (1967).

RESULTS AND DISCUSSION

The purification of polynucleotide ligase was performed exactly as described by Weiss and Richardson (1967); it involves (1) disruption of cells by sonication followed by centrifugation to remove debris, (2) precipitation with 0.2 volumes of 5% streptomycin sulfate, (3) precipitation of the supernatant with ammoniumsulfate (3.3 g per 10 ml), (4) adsorption of the redissolved precipitate to DEAE-cellulose in 0.01 M Tris buffer pH 7.6 followed by desorption with 0.3 M NaCl, (5) readsorption to and elution from DEAE-cellulose with a linear gradient from 0 to 0.3 M NaCl, and (6) adsorption to and elution from phosphocellulose with 0.5 M KCl. Steps 1 to 4 are simple and can be performed in the absence of an enzyme assay. Step 5 requires assaying of the individual fractions. These fractions were tested for ligase activity using both the chemical and the biological assay. As shown in Figure 1, the peaks of ligase activity resulting from the two assays coincide. Across the ligase peak the activity as measured by the chemical assay varies from 10 to 40 units per mg of protein; these activities are comparable to the average of 23 u/mg reported by Weiss and Richardson for their corresponding fraction.

Table 1 shows the result of an experiment in which the transforming activity of nicked r^+ DNA before and after ligase treatment was tested with three different rII deletion mutants as recipients. Nicked DNA transformed the small deletion of mutant r1519 with only slightly reduced efficiency. In contrast, transformation of the double mutant r386/1519 carrying a small deletion near either end of the rII region, as well as of mutant rJ3, a large

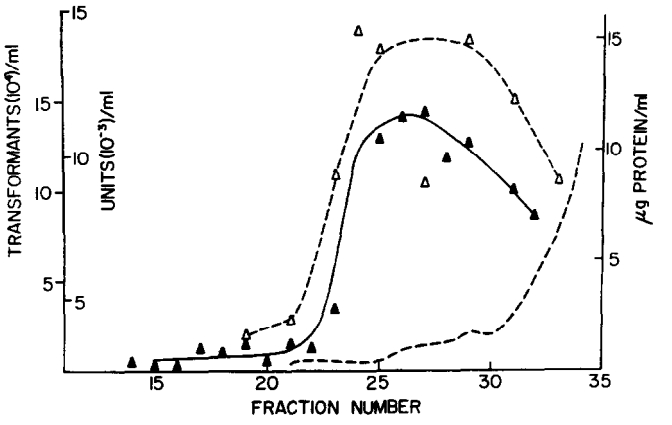

Figure 1: Biological and chemical assay of polynucleotide ligase in fractions obtained on DEAE chromatography of a partially purified extract. Conditions of elution were those of Weiss and Richardson (1967). Equal quantities of enzyme (20 μl of the fractions) were used for the two assays.
▲ and solid line: Polynucleotide ligase activity as determined by the biological assay.
Δ and broken line: Polynucleotide ligase activity as determined by the chemical assay.
Dotted line: protein as determined by the Lowry method after dialysis to remove interfering 2-mercaptoethanol.

TABLE 1

Recovery of transforming activity of nicked DNA upon incubation with polynucleotide ligase (20 μl of a 1:1 dilution). Conditions as in Materials and Methods. The data represent the yield of r^+ transformants per 10^6 rII mutant phage particles.

DNA	Extract	rII mutants used as recipient		
		r1519	r386/1519	rJ3
Unnicked	-	1.11	0.344	0.239
Nicked	-	0.69	0.008	0.005
Nicked	P1*	0.76	0.147	0.067
Nicked	P2	0.93	0.156	0.062
-	P1	0	0	0

*P1 and P2: Phosphocellulose fractions of two different ligase preparations.

deletion spanning the equivalent of slightly more than the rII region, is greatly reduced. Treatment of nicked DNA with polynucleotide ligase under the conditions described in Materials and Methods resulted in a 12 to 15 fold increase in transformation for these two markers, whereas omission of nicked DNA from the incubation mixture yielded no r^+ transformants.

Under the conditions of the assay the nicked DNA remains in an apparent intact helical form and becomes fragmented only after the denaturation step prior to incubation in the transformation assay. Most of these single stranded fragments are still large enough to repair the lesion of the mutant genome r1519 but too small to supply the entire rII region needed to transform the double mutant r386/1519. Repair of the single strand breaks by the polynucleotide ligase results in larger fragments upon denaturation and increased transforming activity. It is clear that the biological assay only detects polynucleotide ligase if the enzyme preparation has more ligase than endonuclease activity. The ligase becomes sufficiently free of nucleases only during the step of DEAE chromatography; the previous steps must therefore be carried out in the absence of an assay. Since this can be done, unavailability of γ-labeled ATP or of polynucleotide kinase does not need to preclude the use of phage induced ligase in studies on DNA-mediated transformation.

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