REPAIR OF SINGLE STRAND BREAKS IN TRANSFORMING DNA BY POLYNUCLEOTIDE LIGASE

Jun Takagi, Tadahiko Ando, and Yonosuke Ikeda
Institute of Physical and Chemical Research, Yamatomachi, Saitama, Japan

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Recently, a few enzymes capable of repairing single strand breaks occurred in a double stranded DNA molecule were reported by several investigators (Weiss and Richardson, 1967, Gefter, et al., 1967, Olivera and Lehman, 1967, Zimmermann, et al., 1967). Bautz (1967) reported a biological study that the single strand damages occurred in phage T4 DNA were repaired by polynucleotide ligase.

We studied the repair of single strand breaks occurred in transforming DNA of Bacillus subtilis by polynucleotide ligase. The breaks were induced by two nucleases, DNase I and DNase K2. DNase K2 is an endonuclease produced by Aspergillus oryzae and isolated at our laboratory (Kato, Ando, and Ikeda, 1967).

MATERIALS AND METHODS

Enzymes. DNase I(IX, cryst.) was purchased from the Worthington Biochemical Corporation. DNase K2 was kindly supplied by Dr. Mikio Kato of this laboratory.

Polynucleotide ligase. Polynucleotide ligase was prepared principally by the method of Weiss and Richardson (1967). The ligase-specific sedimentation pattern was obtained when nicked T4 DNA was treated with the enzyme preparation.

Preparation of nicked DNA. DNA was prepared from a wild type strain of Bacillus subtilis Marburg by the method described by Saito and Miura (1963). When nicked DNA was to be prepared by DNase I, 2 mg of the DNA was treated with 5mμg of DNase I in 2 ml solution consisting of 5 mM $MgCl_2$ and 20 mM Tris-buffer (pH 8.0). The mixture was incubated at 20°C for 30 minutes and, after addition of 0.07 ml of 0.5 M Na-EDTA (pH 8.0), dialysed against 0.01 M Tris-buffer(pH 7.6) supplemented with 0.02 M NaCl. When nicked DNA was to be prepared by DNase K2, a reaction mixture (2 ml) containg 2 mg DNA, 80 mM Tris-buffer (pH 9.0), 20 mM $MgCl_2$, and DNase K2(12 units) was used. The incubation and subsequent treatment were done as described above.

Treatment of nicked DNA with ligase. One hundred μg of the nicked DNA was mixed with polynucleotide ligase (0.04 ml of the DEAE-0.3 M NaCl fraction, Weiss and Richardson, 1967) in 0.4 ml solution consisting of 100 mM Tris-buffer(pH 7.6), 20 mM $MgCl_2$, 20 mM 2-mercapto-ethanol, 0.6 M NH_4Cl, and 66mM ATP. The mixture was incubated at 37°C for 30 minutes and, after addition of 0.01 ml of 0.5 M EDTA, dialysed against 0.01 M Tris-buffer (pH 7.6) supplemented with 0.2 M NaCl.

Transformation procedures. Competent cells of B. subtilis Marburg strain 3115 (his_2 try_2 arg) were exposed to 0.2 or 0.3 μg/ml of native, nicked, or ligase-treated

DNA. The first growth medium described by Takagi, Saito, and Ikeda (1966) was supplemented with 10^{-7} M $MnCl_2$ and the second growth medium with 5 X 10^{-3} M spermine tetrahydrochloride.

RESULTS AND DISCUSSION

Table I shows results of transformation of $\underline{his}_2$-$\underline{try}_2$

Table 1. Repair of DNase I-nicked transforming DNA by polynucleotide ligase.

Exp.	DNA µg/ml	Treatment with DNase I	Ligase	Number of transformants per ml $\underline{his}_2^+$-$\underline{try}_2^+$	%	$\underline{try}_2^+$	%	Co-transfer index
I	0.2	-	-	44,300	142.9	87,600	18.1	0.34
	0.2	-	+	31,000	100.0	74,200	100.0	0.26
	0.2	20 min.	-	6,000	19.4	16,500	22.2	0.22
	0.2	20 min.	+	29,900	96.7	74,300	100.1	0.25
	0.2	40 min.	-	7,100	22.9	20,900	28.1	0.21
	0.2	40 min.	+	21,000	70.0	65,200	87.8	0.20
						$\underline{arg}^+$	%	
II	0.3	-	-			290,000	100.0	
	0.3	6 min.	-			31,000	10.3	
	0.3	6 min.	+			210,000	72.4	
III	0.3	-	-			20,220	100.0	
	0.3	10 min.	-			250	1.2	
	0.3	10 min.	+			3,470	16.0	

Table 2. Repair of DNase K2-nicked transforming DNA by polynucleotide ligase.

Exp.	DNA µg/ml	Treatment with DNase K2	Ligase	Number of transformants per ml $\underline{his}_2^+$-$\underline{try}_2^+$	%	$\underline{try}_2^+$	%	Co-transfer index
IV	0.2	-	-	7,210	100.0	15,500	100.0	0.30
	0.2	30 min.	-	2,775	38.4	5,170	33.3	0.38
	0.2	30 min.	+	6,875	95.3	11,500	74.1	0.43

(linked), $\underline{try}_2$, and arg markers in strain 3115 after exposure to native, DNase I-nicked, or ligase-treated DNA. As the data indicate, native DNA loses its transforming activity by treatment with DNase I and nicked DNA restores its activity by treatment with ligase. If transformation is to be achieved as a result of incorporation of one strand of donor DNA in DNA of recipient strain (Bodmer, 1966), the transformation frequency may be reduced by single strand breaks and enhanced by rejoining. The table also shows that a transforming DNA inactivated by DNase I to 10 - 20 % is reactivated by ligase to 70 - 100 % of the control (the DNA treated solely with ligase), while a DNA inactivated severely by DNase I is not reactivated by ligase to a high percentage. Strange is the result that the joint transformation of $\underline{his}_2$-$\underline{try}_2$ is influenced as equally as the single marker transformation. The reason has not been clarified.

Similar experiments were performed with DNase K2-nicked DNA. The results are shown in Table 2. The $\underline{try}_2$ and $\underline{his}_2$-$\underline{try}_2$ markers are damaged by DNase K2 and repaired by ligase. Since DNases I and K2 are enzymes which split phosphodiester-bonds in DNA strands at the 3′ side, the presence of 5′ terminal phosphate in the nicked DNA, and ATP, may be a requsite condition for rejoining (Weiss and Richardson , 1967).

The data in Tables 1 and 2 suggest that our ligase preparation is contaminated with a small amount of nuclease. A paper will be published elsewhere about the nuclease contained in the preparation.

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