Properties of DNA Polymerases from a Radiation-sensitive Mutant of *Bacillus subtilis*

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Our previous works have demonstrated that the mutant of *Bacillus subtilis* which has the reduced DNA polymerase activity (extracts of the mutant have 15~20% of the wild type activity with calf thymus DNA used as template), is approximately two to three times as sensitive to γ -rays as the wild type strain.¹⁾ The capacity for rejoining the radiation-induced strand-breaks of DNA in the *B. subtilis* mutant is much lower than that in the wild type strain.²⁾ Furthermore, it has been reported by some workers that *B. subtilis*, like *Escherichia coli*, contains three different DNA polymerases.^{3~6)} The major activity, DNA polymerase I seems to be essential for the repair of radiation-damaged regions in the DNA, whereas polymerase III does not. The role of polymerase II is still not clear.

In this paper, we report the characterization of a mutant of *B. subtilis*, which has the reduced DNA polymerase activity and is sensitive to γ -rays.

B. subtilis (Marburg 168, $\text{thy}^- \text{trp}^-$) (168Tt)⁷⁾ was used as parent or wild type strain. Strain D22 ($\text{thy}^- \text{trp}^-$), the mutant of *B. subtilis* 168 Tt having the reduced DNA polymerase activity, was kindly supplied by N. Munakata. Cells were grown in the liquid Schaeffer medium⁸⁾ and harvested at the late logarithmic growing stage.

Partial purification of DNA polymerases was carried out following the procedure described by Ganesan, Yehle and Yu⁵⁾ with slight modi-

FIG. 1. DEAE-cellulose Chromatography of DNA Polymerases from Mutant (D22) and Wild Type (168Tt) Strains of *B. subtilis*.

The activity of DNA polymerase was assayed in 0.5 ml of an incubation mixture containing 65 mM Tris-HCl (pH 8.0), 6.5 mM MgCl_2 , 25 μM dATP, dCTP and dGTP, 5 μM TTP, 0.5 μCi ^3H -TTP (specific activity, 15 Ci/mmol), 25 μg of activated calf thymus DNA and enzyme fraction. The reaction was terminated after 30 min incubation at 37°C by adding carrier yeast RNA and 5% trichloroacetic acid. The precipitates were collected on Whatman GF/C glass fiber filters. The filters were dried and the radioactivity was counted.

(- - -), absorbance at 280 nm; (O—O), DNA polymerase activity; (●—●), DNA polymerase activity in the presence of 5mM NEM; (-----), KCl concentration.

fications. As shown in Fig. 1, DNA polymerase in strain D22 was separated into two distinct fractions (Fractions A and B) on a DEAE cellulose column. Fractions A and B were eluted at 0.20~0.26 M KCl and 0.35~0.45 M KCl, respectively. On the other hand, the chromatogram of DNA polymerase in strain 168 Tt on DEAE cellulose column revealed one major peak, D, one minor peak, C, and a definite shoulder, E, of polymerase activities. The fractions C, D and E were eluted at 0.21~0.26 M KCl, 0.30~0.40 M KCl and 0.40~0.45 M KCl, respectively.

The various fractions and pooled polymerase peaks were assayed to examine further pro-

FIG. 2. Effect of KCl on DNA Polymerase Activity of Various Enzyme Fractions.

(●—●), fraction A; (●---●), fraction B; (○—○), fraction C; (○---○), fraction E.

properties of DNA polymerases (Figs. 1 and 2, and unpublished data). In strain D22 fraction A was sensitive to *p*-hydroxymercuribenzoate (pHMB) and maximally active at about 0.08 M KCl. Fraction B was sensitive to N-ethylmaleimide (NEM) and pHMB, and inhibited at all concentrations of KCl tested. In strain 168 Tt fraction C was sensitive to pHMB and maximally active at about 0.06 M KCl. Fraction E was inhibited by NEM and pHMB and at all concentrations of KCl tested. Fraction D, however, was not inhibited by NEM and pHMB.

Based on the above-mentioned results and some other reports³⁻⁶⁾ on the properties of DNA polymerases, the following conclusion

may be given. The wild type strain 168 Tt possesses at least three different DNA polymerases. Of these, fractions C, D and E seem to correspond to DNA polymerases II, I and III,⁵⁾ respectively. Fractions A and B in the mutant strain D22 probably correspond to DNA polymerases II and III,^{5,6)} respectively. These facts suggest that the difference between the mutant and the wild type strains in regard to response to ionizing radiations can be explained at least partly by the genetic differences in the repairing ability, particularly in the DNA polymerase levels of those strains, considering that DNA polymerase I functions primarily in the repair of radiation-damaged DNA.

Studies on the possible role(s) of DNA polymerases and nucleases in the repair system are now in progress.

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REFERENCES

- 1) H. Terano and H. Kadota, *Bull. Inst. Chem. Res., Kyoto Univ.*, **51**, 59 (1973).
- 2) H. Terano and H. Kadota, *Shokuhin Shōsha*, **18**, 58 (1973).
- 3) M. M. Neville and N. C. Brown, *Nature New Biology*, **240**, 80 (1972).
- 4) G. W. Bazill and J. D. Gross, *ibid.*, **240**, 82 (1972).
- 5) A. T. Ganesan, C. O. Yehle and C. C. Yu, *Biochem. Biophys. Res. Comm.*, **50**, 155 (1973).
- 6) N. R. Cozzarelli and R. L. Low, *ibid.*, **51**, 151 (1973).
- 7) J. L. Farmer and F. Rothman, *J. Bacteriol.*, **89**, 262 (1965).
- 8) I. Takahashi, *ibid.*, **89**, 294 (1965).