SHORT COMMUNICATIONS

Evidence for Indirect Production of DNA Strand Scissions during Mild Heating of Escherichia coli

By S. G. SEDGWICK and B. A. BRIDGES

School of Biological Sciences and MRC Cell Mutation Unit,
University of Sussex, Brighton, BN1 9QH

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Bridges, Ashwood-Smith & Munson (1969a, b) reported a general correlation between sensitivity to mild (52 °C) heating and ionizing radiation among various strains of Escherichia coli. This was confirmed for E. coli and Saccharomyces cerevisiae by Matsumoto & Kagami-Ishi (1970). Bridges et al. (1969b) also demonstrated the production of DNA strand breaks, possibly similar to those produced by ionizing radiation, during exposure of E. coli B/r to 52 °C. They postulated that bacteria used the same processes to repair or mitigate the effects of both types of damage. The cause of the scissions was not established but could include chemical hydrolysis, enzymatic (endonuclease) hydrolysis, and depurination.

Subsequent work showed that the response of Escherichia coli strains to mild heating is greatly influenced by cultural factors not all of which are known. Whereas an unexpected increase in sensitivity of a repair-proficient strain could be attributed to a failure of the repair functions, an increase in resistance of a repair-deficient strain might imply that the primary damage was diminished. We therefore re-examined the effect of mild heating on the molecular weight of DNA from various E. coli bacteria.

We used a modification (Kato & Kondo, 1970) of the alkaline sucrose-gradient technique of McGrath & Williams (1966) which allowed immediate lysis of bacteria on top of the gradient without prior conversion to protoplasts. Bacteria were grown overnight in M9 medium +0.1% (w/v) Casamino acids +20 μg/ml thymine. Subcultures (1 ml) grown for 3 h in the same medium plus 20 μCi/ml [3H]thymidine yielded logarithmically growing bacteria with randomly labelled DNA.

After various periods of incubation at 52 °C, bacteria in 0.1 ml samples of culture were lysed in 0.2 ml 1% (w/v) sodium dodecyl sulphate, 0.25 N-NaOH, 0.005 M-EDTA, 0.05 M-NaCl and 0.005 M-tris layered on top of a 12 ml 5 to 20% linear sucrose gradient (pH 12.2) containing 0.2 N-NaOH and 0.001 M-EDTA. Gradients were centrifuged in a Spinco SW 40 rotor in a L2 65B centrifuge for 3 h at 33,000 rev./min. at 20 °C.

In all experiments the position of the peak for DNA from unheated bacteria was essentially the same. With Escherichia coli K-12, AB1157 and W3110 a decrease in molecular weight was always observed after heating but the kinetics of the process were rather variable. Sometimes there was a delay of up to 20 min before breaks were detected. On other occasions substantial breakage was observed by 20 min, and there was very little further change after 40 min. The radiation-sensitive polA (de Lucia & Cairns, 1969) derivative of W3110 (which is deficient in DNA polymerase I) behaved similarly to the pol+ strain both as regards DNA degradation (Fig. 1a, b) and survival after mild heating. Since pol+ bacteria very rapidly repair many scissions produced by ionizing radiation (Town, Smith & Kaplan, 1971), this
result suggests either that DNA polymerase I may not be active in vivo in some strains at 52 °C, or that the breaks produced during heating are not amenable to polymerase repair.

Two other strains differing in DNA polymerase I activity were also tested, H/r 30-R res+ and R15 resA (Kato & Kondo, 1967, 1970). (The resA gene was isolated in the B family of Escherichia coli, is generally similar to the polA gene, and has the same map position.) R15 has been shown to be more heat-sensitive than H/r 30-R (Bridges et al. 1969a). There was no significant effect of heating at 52 °C on the position of the main peak in either strain (Fig. 1c, d), indicating that breaks were not produced in the bulk of the DNA during heating. There was, however, a consistent loss of acid-insoluble activity as incubation at 52 °C progressed, amounting to about 35 to 45% at 30 min. We interpret this to mean that, before the fragmentation into large pieces which probably occurs at the time of lysis on the gradient, DNA had been degraded from existing ends by removal either of single nucleotides or small oligonucleotides during heating of these particular strains.

Our observations are not readily reconciled with the production of DNA strand degradation by the direct action of elevated temperature by hydrolysis or depurination, as neither of these processes would be expected to be dependent on either the strain or the physiological state of the bacteria. The results are more consistent with attack by nucleases (exo- and/or endonucleases, depending upon the strain and the physiological state of the organisms) released or activated by mild heating. We suggest that, while the production of DNA strand scissions may play an important role in the lethal effect of mild heating in certain circumstances, it can not be assumed to be a general phenomenon.
REFERENCES


