THE EFFECT OF THYMINE STARVATION ON CHROMOSOMAL STRUCTURE OF ESCHERICHIA COLI JG-151

David L. Reichenbach, George E. Schaiberger and Bennett Sallman

Department of Microbiology
School of Medicine
University of Miami, Miami, Florida 33136

Received November 12, 1970

Summary

Labelled DNA extracted from control and thymine starved cells was qualitatively characterized with respect to sedimentation properties in alkaline sucrose gradients. DNA isolated from cells undergoing loss of division ability demonstrated decreasing sedimentation velocity. Sedimentation profiles of DNA extracted from cells which were starved for thymine under conditions which allowed spontaneous recovery of division ability to occur, demonstrated an increase in DNA sedimentation velocity toward normal control value. It appears that while thymine starvation can result in single strand breaks, this damage is not irreversible, for under certain conditions rejoining of the breaks can occur.

The question of what happens to DNA during thymine starvation is not only an extremely interesting one but also one which must be resolved before the mechanism of thymineless (TD) death can be understood. McFall and Magasanik (1962) (1) were the first to suggest that periods of thymine deprivation could damage DNA. Indeed, there is a good deal of circumstantial evidence that implies that some alterations do occur in bacterial DNA upon thymineless incubation (2,3,4,5,6,7). There are however, several lines of evidence that clearly seem to rule out the possibility that extensive fragmentation of DNA is necessarily associated with thymineless starvation (8,9,10,11).

The first definitive experiments describing DNA damage during thymine starvation were reported by Freifelder (1969) using an extra-chromosomal DNA system(12). This communication presents evidence that chromosomal DNA is damaged during periods of thymineless incubation thru the introduction of single strand nicks. Furthermore, under certain conditions these strand breaks were found to undergo repair.

Previous work by others in this laboratory had shown that the viability kinetics of E. coli JG-151 (a colicin cured derivative of E. coli $15T^-$) could be manipulated (13,14). Under certain conditions JG-151 demonstrated typical loss of viability during the first 150 minutes of thymine starvation; at this point however, these cells spontaneously regain division ability or recovery (Figure 1, solid line). Other conditions, predispose the same strain to loss of division ability thru the first 150 minutes of thymineless deprivation followed by no further change in viability (Figure 1, broken line). The important parameter involved here was found to be the ratio of cell culture volume to the volume of the flask in which the culture was incubated during thymine starvation. Cell death and recovery could be simply and reproducibly manipulated by merely varying the size of culture volume. This system employing JG-151 (t^-,col^-) therefore, offered a unique opportunity for the study of the effects of intrinsic thymineless death on DNA structural integrity. In sedimentation analysis experiments, labelled DNA extracted from cells incubated under conditions which allowed only TD to occur (Figure 1, dotted line) were compared to cells incubated under conditions which allowed recovery to occur (Figure 1, solid line). It was possible to extract and compare DNA from cells which had experienced identical growth, labelling, and manipulatory events but whose fate during identical periods of thymineless starvation was markedly different.

MATERIALS AND METHODS

The strain employed throughout the study was E. coli JG-151, a colicin cured derivative of E. coli $15T^-$ (15). All experiments

were performed in M9 minimal medium (16).

Thymineless conditions were obtained by centrifuging rapidly growing cell cultures (o.d. 0.24-.26 at 420 mμ) for 10 minutes, 15° C, 2520 rcf. The pelletized cells were resuspended in M9 containing no glucose or thymine and centrifuged as above. The resulting sediment was resuspended in 10 ml of M9; this dense suspension serving as the inoculum for thymineless flasks containing M9 and glucose. The volume of M9 was such that the resulting cell suspension had an optical density of 0.24. For thymineless incubation the glucose concentration used was 0.6%.

Cells fully labeled in C-14 thymine were transformed into protoplasts by a modification of the lysozyme-versene method (17). Approximately 4. x 10^8 cells were suspended in 0.3 ml of TEP buffer (18) at pH 8.1, and 0.06 ml. of 30% sucrose in 0.6 M Tris pH 8.1 was added. After gently mixing 0.04 ml of a 10% lysozyme solution in 0.25 M Tris (pH 8.1) was added. This was followed by the addition of 0.1 ml of 0.032 M EDTA. The resulting solution was placed in a 0° C. water bath for 5 minutes to allow protoplast formation. The protoplasts (approximately 1 x 10^7) were then lysed by pipetting them slowly on a 0.12 ml layer of 0.5 N NaOH which had been placed on top of a 7 - 20% alkaline (pH 12.0) sucrose gradient. Gradients were centrifuged at 30,000 rpm for 90 minutes at 20° C. in the SW-39L swinging bucket rotor of a Beckman Model L. ultracentrifuge. Contents of the gradients were collected in fractions of four drops each using an ISCO model 180 density gradient fractionator. Fractions were collected directly into scintillation vials, suspended in an aqueous scintillant system (5.5% Liquifluor, 4.0% Beckman Bio-solv, 90.5% toluene), and assayed for radioactivity in a 3375 Packard Tri-Carb Liquid Scintillation Spectrometer. Sedimentation constant values were computed thru the integration of the equation of Martin and Ames (19). $S_{20,w}$ values were then substituted into the equation derived by Studier (20) relating $S_{20,w}$ to molecular weight for alkaline DNA.

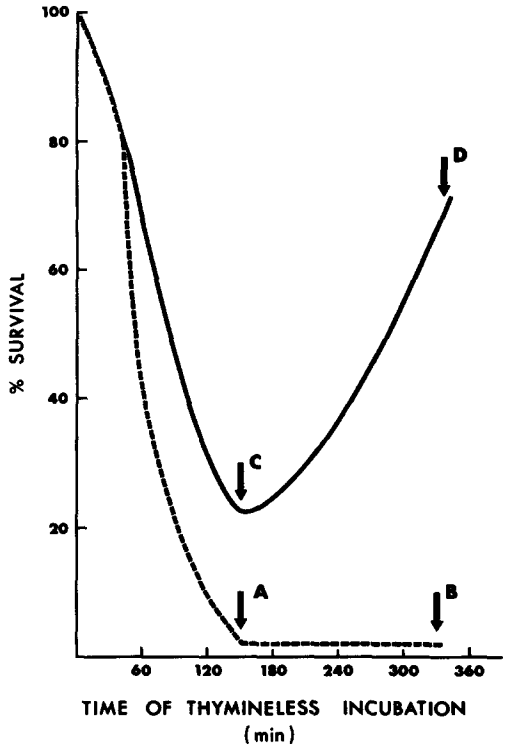

Figure 1. A comparison of the viability curves of a cell population undergoing TD (broken line) with an identical population undergoing TD followed by recovery (solid line). The ratio of cell culture volume to flask volume was 0.4 for recovering cells and 0.08 for the cells undergoing TD without recovery. The points at which cell samples were taken for DNA extraction and subsequent sedimentation analysis are indicated.

RESULTS AND DISCUSSION

Figure one illustrates the viability kinetics which are produced when JG-151 is subjected to thymine starvation under two different sets of conditions: conditions which lead to TD (Figure 1, broken curve), and conditions which produce TD followed by recovery (Figure 1, solid curve). Maximum loss of viability occurred after 150 minutes of starvation in both cases (Figure 1, Points A & C). Under TD conditions no further change in viability was seen upon prolonged starvation. Under recovery conditions, division ability increased after 150 minutes of thymine

deprivation and rose to involve greater than 70% of the original cell population. The arrows indicate the time points at which DNA was extracted from the respective cell cultures and subjected to sedimentation analysis. Figure 2 compares the sedimentation profiles of control DNA (DNA extracted from log phase cells not subjected to starvation) with the sedimentation profiles produced by cells harvested 150 minutes and 330 minutes after the onset of thymineless incubation. The average molecular weight values

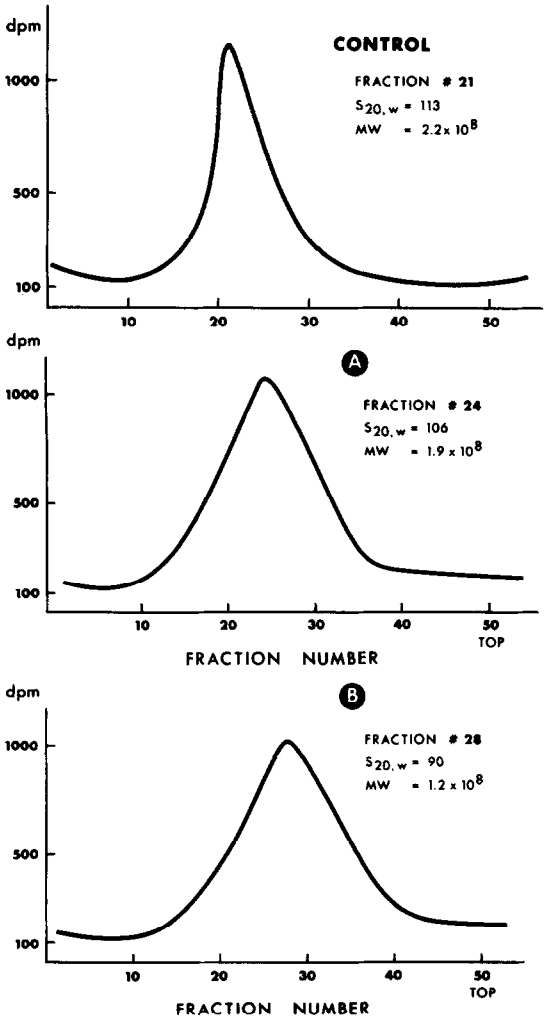

Figure 2. A comparison of the sedimentation profiles of DNA extracted from control cells with DNA extracted from cells after 150 minutes of TD (Curve A) and 330 minutes of TD (Curve B).

for the 150 minute sample and the 330 minute sample were 1.85 x 10^8, and 1.2 x 10^8 respectively, compared to a value of 2.2 x 10^8 for control DNA. The results of this study indicate that single-strand nicks occur in the DNA of this thymine requiring mutant during periods of thymine deprivation. Furthermore, the number of nicks appears to increase upon prolonged starvation.

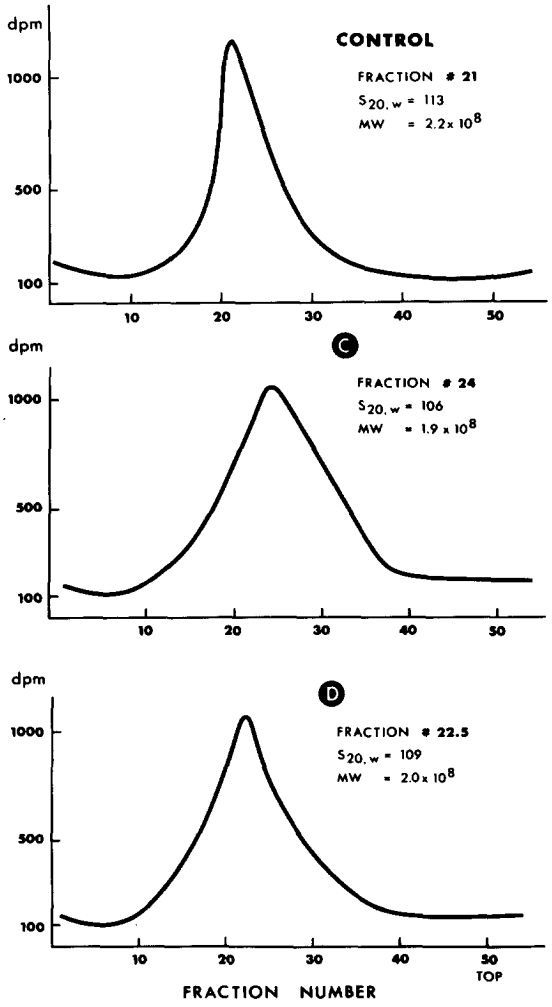

Figure 3. A comparison of the sedimentation profiles of DNA extracted from control cells with DNA extracted from cells starved for thymine under recovery conditions. Curve C 150 minutes of starvation, Curve D 330 minutes of starvation.

Figure 3 compares the sedimentation profiles of DNA extracted from cells after 150 minutes (point C) and 330 minutes (point D) of thymineless incubation under conditions allowing recovery with the sedimentation profile for control DNA. The average molecular weight of DNA extracted from cells after 330 minutes of starvation under recovery conditions was higher than the similar value for DNA extracted from the same cells at 150 minutes of thymineless incubation, and approaches that of control DNA.

These data indicate that the average number of single strand breaks in the DNA decreases during recovery indicating repair. This repair process does not require nucleotides since DNA is extracted from cells which are harvested directly from the thymineless conditions. The repair seen here may involve a simple rejoining of a break in the DNA backbone.

ACKNOWLEDGMENTS

David L. Reichenbach is a USPHS predoctoral trainee supported by grant 1 T01 HD 00142-04,and George E. Schaiberger the recipient of USPHS Career Development Award K3-HD-34945. This work was supported in part by grant IN-51J from the American Cancer Society.

REFERENCES

1. McFall, E., Magasanik, B., Biochimica et Biophysica Acta, 55, 920, 1962.
2. Coughlin, C., and Adelberg, E., Nature, 178, 531, 1956.
3. Pritchard, R., and Lark, K., J. Mol. Biol., 9, 288, 1964.
4. Pauling, C., Hanawalt, P., Proc.Nat.Acad.Sci.,57,1426,1965.
5. Gold, M., and Horwitz, J., Cold Spring Harb. Symo. Quant. Biol., 28, 149, 1963.
6. Schaiberger, G., Giegel, J., and Sallman, B., Biochem. Biophys., Res. Commun., 28, 30, 1967.
7. Mennigmann, H.D., and Szybalski, W., Biochem. Biophys. Res. Commun., 9, 398, 1962.
8. Nakada, D., and Ryan, F.J., Nature, 189, 398, 1961.
9. Luzzati, D., and Revel, C., Biochem. Biophys. Acta, 61, 305, 1962.
10. Smith, B.J., Burton, K., Biochem. J., 97, 290, 1965.
11. Freifelder, D., Maaloe, O., J. of Bact., 88, 987, 1964.
12. Freifelder, D., J. Mol. Biol., 45, 1, 1969.
13. Sallman, B., de Velasco, F., Bact. Proc. GP82, 60, 1968.

14. Donachie, W.D. and Hobbs, D.G. Biochem. Biophys. Res. Commun. 29, 172-176, 1967.
15. Ishibashi, M., Hirota, Y., J. Bact., 90, 1496, 1965.
16. Lark, K., Repko, T., and Hoffman, E., Biochim. Biophys. Acta., 76, 9, 1963.
17. Fraser, D., Mahler, R., Shug, A., and Thomas, C., Proc. Nat. Acad. Sci., 43, 939, 1957.
18. Billen, D. Personal communication. Laboratory of Radiation Biology, Univ. of Florida, Gainesville, Florida.
19. Martin, R., Ames, B. J. Biol. Chem., 236, 1372, 1961.
20. Studier, W., J. Mol. Biol., 11, 373, 1965.