CIRCULAR DICHROISM AND THERMAL DENATURATION STUDIES OF CHROMATIN AND DNA FROM BrdU-TREATED MOUSE FIBROBLASTS

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Summary: DNA and chromatin were isolated from control 3T6 mouse fibroblasts and from 3T6 grown in 72 μM 5-Bromodeoxyuridine (BrdU). BrdU-containing DNA showed a blue shift in the 250-300 nm region of circular dichroism spectra and an increase in melting temperature over control DNA. These changes were even more accentuated in chromatin, suggesting an altered interaction between chromosomal proteins and BrdU-containing DNA.

5-Bromo-2'-deoxyuridine (BrdU) can be incorporated in place of thymidine into the DNA of bacteria (1-3) and of mammalian cells either in culture (4,5) or *in vivo* (6,7). BrdU suppresses the phenotypic expression of a wide variety of cell types (8-11, and see review by Rutter *et al.*, 12). In most instances, the effect of BrdU on mammalian cells is due to its incorporation into DNA (13,14). It has recently been demonstrated by Hill and Baserga (15) that replacement of approximately 20% of the thymine residues of DNA from mouse 3T6 fibroblasts by BrdU causes an increased incorporation of GMP and a decreased incorporation of AMP into RNA. The transcriptional error was more pronounced when using chromatin as the template than when using DNA, and, on this basis, these authors concluded that the alteration in the templating activity of DNA was enhanced by chromosomal proteins. We now report that in addition to these functional alterations, BrdU induces structural modifications of DNA as indicated by changes in circular dichroism spectra and thermal denaturation, and these changes seem to be augmented in chromatin.

Methods and Materials

3T6 mouse fibroblasts were grown, as previously described (15,16) in plastic Falcon flasks (75 cm^2). Treatment with BrdU was begun one day after plating by addition of BrdU (Calbiochem) to each flask to a final concentration of 50 μg/ml (72 μM). Cells were confluent at 5 days and were used at 7 days after plating. Significant incorporation of BrdU into DNA under these conditions was indicated by a shift of the buoyant density of DNA in a $CsSO_4$ gradient from a control value of 1.44 g/ml to a density of 1.50-1.51 g/ml (not shown). Replacement of thymine residues by BrdU was estimated to be approximately 15%.

Chromatin was isolated from control and BrdU-treated cells as previously described (17). Briefly, nuclei were prepared by washing cells in 80 mM NaCl, 20 mM EDTA, pH 7.4, containing 1% Triton X-100. The nuclei were lysed in distilled water, layered over 1.7 M sucrose, and the chromatin pelleted at 100,000 x g for 80 minutes. Circular dichroism was measured using a Jasco Model J-40 recording spectropolarimeter. The instrument was standardized as described by Simpson and Sober (18). All experiments were carried out at 23°C in nitrogen atmosphere, in a fused quartz 1 cm cell, using a DNA residue concentration of 1.5×10^{-4} M. The time constant was 4 seconds, scanning speed below 1 cm/min, dynode voltage not over 400 volts, and the precision in band wavelength was 0.3 nm. The mean ellipticity (θ) is expressed in degree cm^2 per dmole of nucleotide residue, assuming the mean molecular weight of a nucleotide as 330. The DNA concentration of chromatin was determined by UV absorption at 260 nm in 0.2% sodium dodecyl sulfate, using an ε of 21,000 cm^2/g.

Absorption melting profiles were measured with a Gilford multiple sample recording spectrophotometer Model 2000. Samples in stoppered cuvettes of 1 cm light path were heated linearly at the rate of 0.5°/min with a Haake temperature regulator. Thermal denaturation of DNA was monitored by absorption changes of 260 nm and hyperchromicity (H_{260}) relative

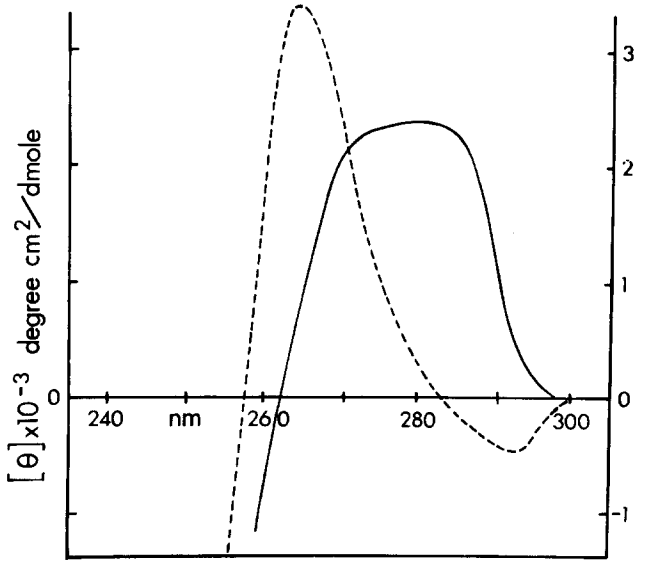

Fig. 1. Circular dichroism spectra of chromatin from untreated 3T6 mouse fibroblasts (——) and BrdU-treated 3T6 cells (----). The solvent was standard saline citrate (SSC) x 0.01 solution.

to absorption at room temperature was calculated degree per degree.

Results

At a BrdU concentration of 72 μM and under present experimental conditions, 3T6 cells remain viable, although growth is reduced in respect to control cells (15). Circular dichroism spectra and melting profiles were obtained for both DNA and chromatin from both control and BrdU-treated cells.

As seen in Fig. 1, the circular dichroism (CD) spectrum of chromatin from BrdU-treated cells shows a blue shift of 12 nm in the 250-300 nm region where alterations in DNA structure can be detected. There are no changes in the α-helix content of proteins as indicated by identical CD spectra of control and BrdU chromatin in the 200-250 nm region (not shown). Consistent with this, free DNA from BrdU cells prepared by the Marmur procedure (19) shows (Fig. 2) an identical blue shift of 12 nm in the 250-300 nm range. However, it should be noted that there is an enhancement of the positive ellipticity at 264 nm and the appearance of a negative band at

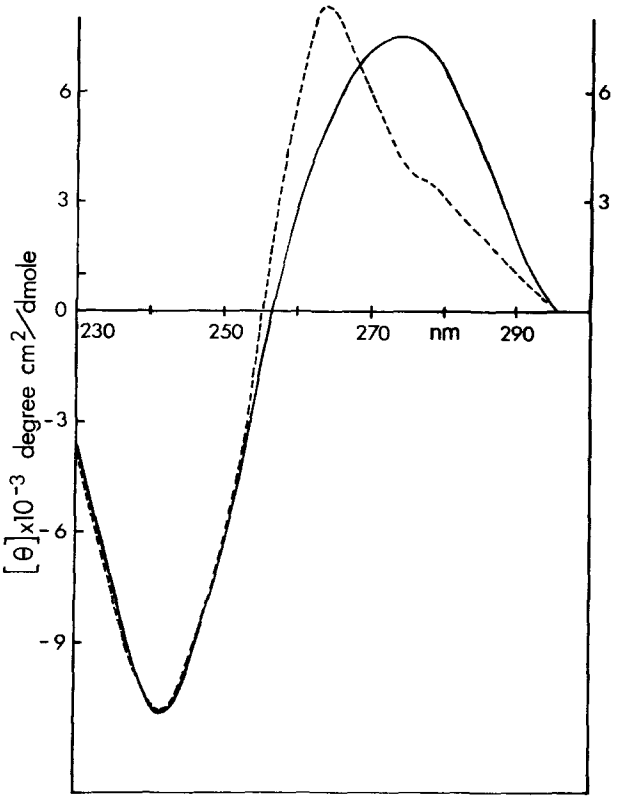

Fig. 2. Circular dichroism spectra of DNA from untreated 3T6 mouse fibroblasts (——) and BrdU-treated 3T6 cells (----). The solvent was standard saline citrate (SSC) x .01 solution, pH 7.3. Circular dichroism was measured as described in Methods and Materials, using a DNA residue concentration of 1.21 x 10^{-4} M.

293 nm in BrdU chromatin (Fig. 1) that are not seen in BrdU-DNA (Fig. 2).

To further characterize the physico-chemical alterations induced by BrdU, melting profiles were done on chromatin and DNA. Fig. 3 shows that the melting temperature (T_m = 73°C) of BrdU protein-free DNA, in Standard Saline Citrate (SSC) x 0.01 solution, is 6°C higher than the melting point of DNA from untreated cells (T_m = 67°C). A similar increase in T_m was seen in BrdU chromatin, as illustrated in Fig. 3a.

To insure that the changes in CD and T_m of BrdU DNA are not due to small amounts of proteins still present after Marmur extraction, DNA from control and BrdU-treated cells was further purified by banding in $CsCO_4$

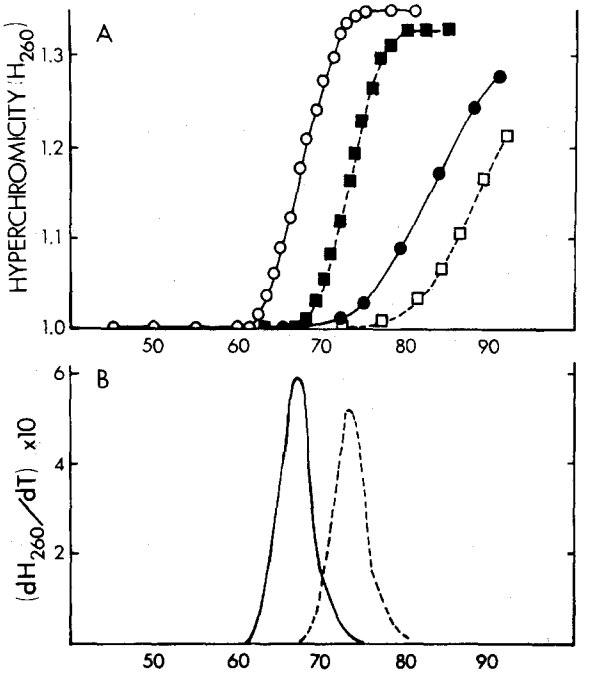

Fig. 3. Thermal denaturation profiles of DNA from untreated (o——o) and BrdU-treated mouse fibroblasts (■----■). The thermal denaturation profiles of chromatin are also shown: 1) from untreated (●——●——●), 2) from BrdU-treated mouse fibroblasts (□----□). The solvent was standard saline citrate (SSC) x 0.01 solution, pH 7.3. The derivative curve calculated, degree by degree, for hyperchromicity increment per degree at 260 nm, is represented in the lower panel, for DNA from untreated (——), or BrdU-treated mouse fibroblasts (----).

according to the technique of Sato et al. (20). This highly purified DNA exhibited the same changes in CD and thermal denaturation as shown in Figs. 2 and 3 (not shown).

Discussion

These results suggest that the substitution of BrdU for thymidine in DNA profoundly affects its structure. The changes in CD spectra of BrdU-substituted DNA are not surprising, although, to our knowledge, they have not been previously reported. An increased T_m in synthetic polynucleotides containing 5-substituted pyrimidine nucleotides has been previously reported by Massoulié et al. (21), who concluded that major factors influencing stability of nucleic acids do not include hydrogen bonding.

However, more important in our results is the observation that the DNA changes caused by BrdU seem to be somewhat augmented by the presence of chromosomal proteins. This is of interest since substitution of thymine by BrdU in E. coli DNA has been shown to dramatically change the affinity of lac repressor for its operator gene (22). It should also be noted that a similar increase in T_m of BrdU chromatin has been reported [cited by Rutter et al.(12)], although these authors did not report differences in free DNA. The finding of Hill et al. (15) that BrdU causes a transcriptional error in the template activity of DNA and that this error is enhanced in chromatin also agrees with the present results and seems to confirm the conclusions of Walther et al. (13) that the interaction of chromosomal proteins with BrdU-substituted DNA is altered.

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References

1. Zamenhof, S., and Griboff, G. (1954) Nature 174, 306-307.
2. Dunn, D. B., and Smith, J. D. (1954) Nature 174, 305-306.
3. Dunn, D. B., and Smith, J. D. (1957) Biochem. J. 67, 494-506.
4. Eidinoff, M. L., Cheong, L., and Rich, M. A. (1959) Science 129, 1550-1551.
5. Cheong, L., Rich, M. A., and Eidinoff, M. L. (1970) J. Biol. Chem. 235, 1441-1447.
6. Hill, B. T., Augenlicht, L., and Baserga, R. (1973) FEBS Lett. 37, 298-302.
7. Gross, N. J., and Rabinowitz, M. (1969) J. Biol. Chem. 244, 1563-1566.
8. Kajiwara, K., and Mueller, G. C. (1964) Biochim. Biophys. Acta 91, 486-493.
9. Davidson, R. L., and Bick, M. D. (1973) Proc. Natl. Acad. Sci. 70, 138-142.
10. Coleman, J. R., Coleman, A. W., and Hartline, E. J. H. (1969) Develop. Biol. 19, 527-548.
11. Stockdale, F., Okazak, K., Nameroff, M., and Holtzer, H. (1964) Science 146, 533-535.
12. Rutter, W. J., Pictet, R. L., and Morris, P. W. (1973) Ann. Rev. Biochem. 42, 601-646.
13. Walther, B. T., Pictet, R. L., David, J. D., and Rutter, W. J. (1974) J. Biol. Chem. 249, 1953-1964.
14. Mochan, B. S., Mochan, E., and De La Haba, G. (1974) Biochim. Biophys. Acta 335, 408-423.
15. Hill, B. T., Tsuboi, A., and Baserga, R. (1974) Proc. Natl. Acad. Sci. 71, 455-459.

16. Tsuboi, A., and Baserga, R. (1972) J. Cell. Physiol. 80, 107-118.
17. Augenlicht, L., and Baserga, R. (1973) Arch. Biochem. Biophys. 158, 89-96.
18. Simpson, R. T., and Sober, H. A. (1970) Biochemistry 9, 3103-3109.
19. Marmur, J. (1961) J. Mol. Biol. 3, 208-218.
20. Sato, S., Ariake, S., Saito, M., and Sugimura, T. (1972) Biochem. Biophys. Res. Commun. 49, 827-834.
21. Massoulié, J., Michelson, A. M., and Pochon, F. (1966) Biochim. Biophys. Acta 114, 16-26.
22. Lin, S., and Riggs, A. (1972) Proc. Nat. Acad. Sci. 69, 2574-2576.