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MUTAGEN-INDUCED CHANGES IN CELLULAR DEOXYCYTIDINE TRIPHOSPHATE AND THYMIDINE TRIPHOSPHATE IN CHINESE HAMSTER OVARY CELLS*

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SUMMARY: Deoxynucleoside triphosphate concentrations in Chinese hamster ovary cell lines, CHO-K1 and Mut 8-16, were examined following exposure of cells to UV or dimethylsulfate. Marked decreases in dCTP were observed 2 hr after exposure to both mutagens. In contrast, dTTP concentrations increased with increased cell killing after exposure to UV but not after exposure to dimethylsulfate. Examination of DNA synthesis in permeabilized cells in the presence of excess concentrations of dNTP substrates suggests that excess dCTP enhances replication while excess of dTTP inhibits replication. We therefore ask whether the increase in the dTTP/dCTP ration in mutagenized whole cells either contributes to or prolongs induced inhibition of replication. In addition we proposed that such an induced dNTP imbalance may also contribute to an increase in mutations by enhancing the probability for base-misincorporation.

Numerous studies have demonstrated that exposure of mammalian cells to UV radiation results in transient inhibition of DNA synthesis (1). The nature of this inhibition is not yet clear. In particular, there remain questions of whether initiation or elongation are principally affected (2-6), to what extent lesions in DNA represent blocks to replication and what factors affect the duration of the recovery period (1,3,5-7).

We have recently studied the effects of cellular exposure to UV or to dimethylsulfate on mutagen-induced changes in dNTP pools in Chinese hamster ovary cells, as described below. We report that significant changes from steady-state occur in cellular concentrations of dCTP and dTTP which might affect

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or prolong mutagen-induced inhibition of replication. We also believe that such an induced pool imbalance may enhance mutagenesis by causing base-misincorporation, in accordance with the report of Kunkel and Loeb (8) for mismatched base pairing by mammalian DNA polymerases in the presence of a dNTP imbalance.

MATERIALS AND METHODS

CHO-K1, its UV-resistant, dimethylsulfate-resistant derivative, Mut 8-16, and growth of monolayers in Ham's F-12 with 10% fetal calf serum have been described earlier (9,10). Briefly, the mutant is resistant to UV and dimethylsulfate, and is normal with respect to excision-repair, post-replication-repair and unscheduled DNA synthesis (9). The only demonstrable defects of Mut 8-16 are a reduced dCTP pool (∿2/3 that of CHO-K1) and an enlarged dTTP pool (2- to 3-fold that of CHO-K1), as described earlier (10).

dNTP pools in growing cultures were assayed enzymatically essentially according to the procedure of North et al. (11). Mutagenized cells were assayed 2 hr after exposure to UV or after a 2 hr exposure to dimethylsulfate, as described in detail elsewhere (10). DNA synthesis in permeabilized cells was examined according to a slight modification of the method of Berger et al. (12), as also described previously (10). The standard reaction mixture contained (in 0.3 ml) 33 mM Hepes, pH 7.8, 6.6 mM $MgCl_2$, 70 mM Nacl, 5 mM ATP, 0.1 mM each of dGTP, dCTP and dATP, 0.73 μM[^{3}H)dTTP (spec. act. x.4 x 10^6 dpm/nmol) and ∿4 x 10^5 permeable cells. Under the conditions employed rates of [^{3}H]dTTP incorporation at 37°C (3-3.7 x 10^{-3} dpm/cell min^{-1}) were linear for at least 30 min (10).

RESULTS AND DISCUSSION

In Figure 1, relative changes from steady-state concentrations of dTTP and dCTP in CHO-K1 and its mutagen-resistant derivative, Mut 8-16 (9) following exposure to UV or dimethysulfate are illustrated. For comparison of the two agents and both cell lines, results are plotted against respective cytotoxic responses. A 3- to 5-fold reduction from steady state concentrations of cellular dCTP occurs, even at high survival (>85%) doses of both mutagens in both cell lines. In contrast, a much enlarged dTTP pool occurs after exposure to UV (at 15 Jm^{-2}, up to 3.7-fold in CHO-K1 and up to 2.2-fold

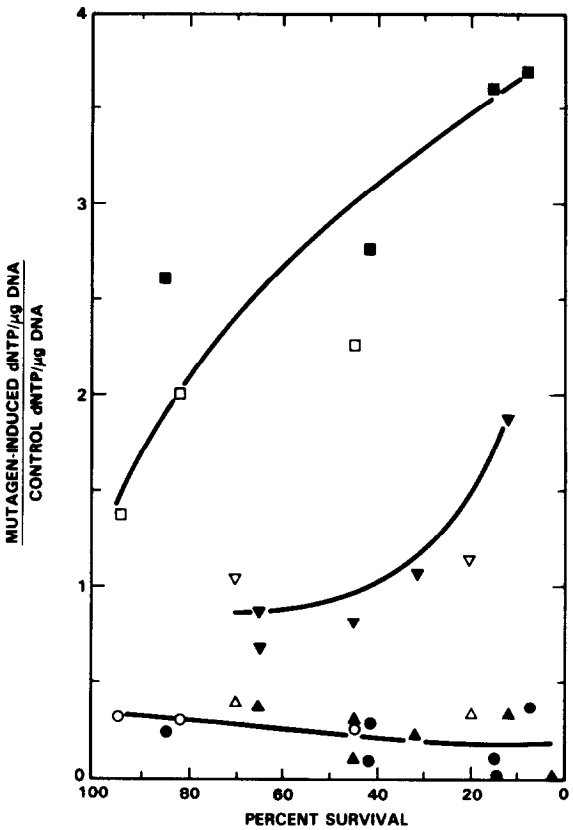

Fig. 1. UV- or Dimethylsulfate-Induced Changes in Relative Concentrations of dTTP and dCTP. Cell monolayers were exposed to UV then incubated in fresh growth medium for 2 hr or cells were grown in the presence of dimethysulfate for 2 hr. The concentrations of dNTP pools in extracts from treated cells were compared to that from untreated control cultures. Survival values after UV or dimethysulfate exposure were obtained earlier (9,10). Relative dTTP: after UV, (■,□) or after dimethysulfate, (▼,▽); relative dCTP: after UV, (●,o), or after dimethylsulfate (▲,△). Closed symbols are CHO-K1; open symbols are Mut 8-16.

in Mut 8-16). To a first approximation data points relating relative increases in dTTP with decreases in survival in both cell lines share a common line. Dimethylsulfate-induced increases in dTTP were considerably smaller and were seen only when survival levels were below 30%. No comparable deviations from steady-state concentrations were observed in dATP or dGTP (not shown).

The impact of an induced dNTP imbalances on DNA synthesis in unperturbed and mutagenized cell cultures is not yet

apparent and must await detailed characterization of replication in dNTP pool-altered cell lines, such as those described by Nicander and Reichard (13), Meuth et al. (14) and Meuth (15). In the interim, we have chosen to examine the affects of altered dNTP concentrations on DNA synthesis in permeable CHO cells, though we acknowledge that such DNA synthesis may not fully reflect that which occurs in intact cells (16). Indeed, we estimate that average rates of synthesis in our permeable cells fall between 10-20% that occurring in unperturbed cells (not shown).

In Figure 2, rates of DNA synthesis in permeable cells in the standard reaction mixture (10,12) here assigned a value of unity, are compared to rates obtained under conditions of singularly increasing the concentrations of each of the four dNTP substrates. No effect on the rate of synthesis was observed with up to a 22-fold increase in the concentration of dATP and only a small (and equivalent) stimulation of synthesis was obtained in both cell lines when dGTP was increased by the same magnitude. In contrast, a dramatic effect on the replication rate resulted when excess dTTP or excess dCTP was added to the standard mixture. A 20-fold excess in dTTP inhibited synthesis to ∿5% of normal in both cell lines, while on the contrary, a 23-fold excess in dCTP enhanced synthesis up to two-fold. The effect of dCTP was most dramatic in CHO-K1 cells; little stimulation of replicative activity in Mut 8-16 was observed at or below dCTP concentrations of 1 mM (10-fold excess) compared to a 1.4-fold increase in parent cells. Judging from the absence of response or small response of both cell lines to excess dATP and dGTP respectively, substrate concentrations were not otherwise rate-limiting. This difference in response to excess dCTP by Mut 8-16 is presently

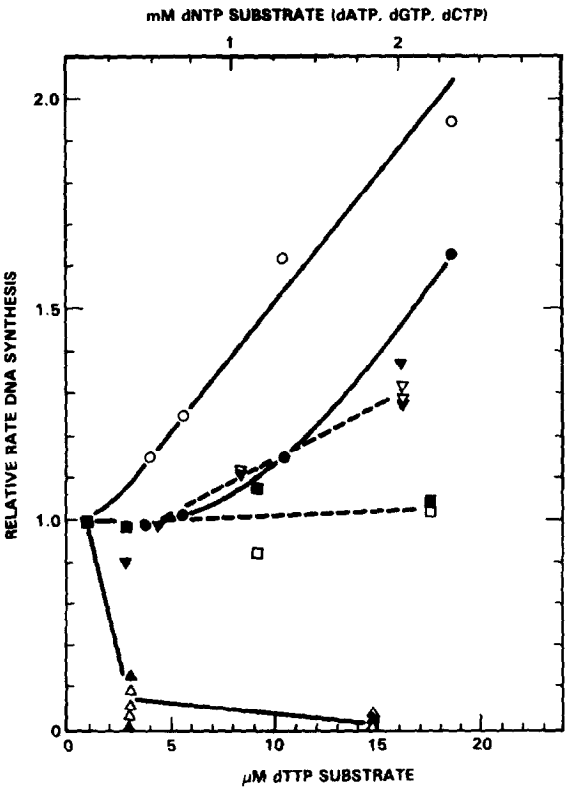

Fig. 2. Effects of dNTP Substrate Concentrations on Rate of DNA Synthesis in Permeable Cells. Rates of DNA synthesis were determined in the standard reaction mixture (relative rate = 1.0) or in the standard mixture containing excess dCTP (●,o), dGTP (▼,▽), dATP (■,□) or dTTP (▲,△) at the indicated concentrations. [^{3}H]dTTP was maintained at a constant specific activity (3.4 x 10^6 dpm/nmol). Open symbols are permeable CHO-K1 cells; closed symbols are permeable Mut 8-16 cells.

an enigma and, along with the unusual pool imbalance in this cell line, may be related to its increased resistance to UV and to alkylating agents (9,10).

It thus appears that replication in permeable cells is stimulated by excess dCTP and inhibited by excess dTTP. The results with dCTP are consistent with the hypothesis of Nicander and Reichard (13) that in unperturbed cells, dCTP may bind to an allosteric site on the replicase and that such binding positively regulates the affinity of the system for all dNTP substrates. The possibility that excess dTTP likewise inhibits replication in whole cells must await further experi-

mentation. It is important to determine whether some component of induced DNA synthesis inhibition, such as at initiation (2,3,5) elongation (5,6) or recovery after prolonged blockage of replication forks at DNA lesions (1,4,5,7) is affected by an induced dTTP/dCTP ratio imbalance (Figure 1) which favors inhibition of the replicase.

Finally, one must also consider the consequences of an induced dNTP imbalance on mutation induction. Kunkel and Loeb (17,18) and Kunkel et al. (19) have clearly demonstrated that changes in relative and absolute dNTP (and deoxynucleoside monophosphate) concentrations increase the frequency of base misincorporation by both procaryotic and eucaryotic DNA polymerases. Peterson et al. (20), Ashman and Davidson (21) and Meuth (15), have each shown that abnormal dNTP pool concentrations in mammalian cells enhance mutation frequencies, presumably because a pool bias favors decreased fidelity (17-19). For example, Ashman and Davidson (21) found that exposure of Syrian hamster melanoma cells to BrdUrd increased the mutation frequency not as a function of the percent substitution for thymine in DNA but as a consequence of a decrease in cellular dCTP. This decrease occurred because the resulting enlarged BrdUrd triphosphate pool inhibited reduction of CDP by ribonucleotide reductase. Addition of CdR to cells in the presence of otherwise mutagenic concentrations of BrdUrd prevented both the decrease in cellular dCTP and BrdUrd-induced mutagenesis.

In conclusion, the significance of our findings is at present only speculative and, therefore, in need of further examination. We are hopeful that future studies will better elucidate both the mechanism and biological impact of mutagen-induced dNTP pool imbalances in mammalian cells.

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REFERENCES

1. Edenberg, H. (1983) Comments Mol. Cell. Biophy. 1, 383-397.
2. Kaufmann, W. and Cleaver, J. (1981) J. Mol. Biol. 149, 171-187.
3. Povirk, L. and Painter, R. (1976) Biophys. J. 16, 883-889.
4. Rudé, J. and Friedberg, E. (1977) Mut. Res. 42, 433-442.
5. Dahle, D., Griffiths, T. and Carpenter, J. (1980) Photochem. Photobiol. 32, 157-165.
6. Doniger, J. (1978) J. Mol. Biol., 120, 433-446.
7. Cleaver, J., Thomas, G. and Park, S. (1979) Biochim. Biophys. Acta 564, 122-131.
8. Kunkel, T. and Loeb, L. (1981) Science 213, 765-767.
9. Newman, C. (1982) Mut. Res. 96, 99-108.
10. Newman, C. and Miller J. (1983) in: Proceedings of the Conference on Genetic Consequences of Nucleotide Pool Imbalance, In Press, Plenum Press, New York.
11. North, T. Bestwick, R. and Mathews, C. (1980) J. Biol. Chem. 255, 6640-6645.
12. Berger, N., Kaichi, A., Steward, P., Kleuecz, G., Forrest, G. and Gross, S. (1978) Exptl. Cell Res. 117, 127-135.
13. Nicander, B. and Reichard, P. (1981) Biochem. Biophys. Res. Commun. 103, 148-155.
14. Meuth, M., Aufreiter, E. and Reichard, P. (1976) Eur. J. Biochem. 71, 39-43.
15. Meuth, M. (1981) Somat. Cell Genet. 7, 89-102.
16. Mattern, M. and Painter, R. (1979) Biochim. Biophys. Acta 563, 293-305.
17. Kunkel, T. and Loeb, L. (1979) J. Biol. Chem. 254, 5718-5725.
18. Kunkel, T. and Loeb, L. (1981) Science 213, 765-767.
19. Kunkel, T., Schaaper, R., Beckman, R. and Loeb, L. (1981) J. Biol. Chem. 256, 9883-9889.
20. Peterson, A., Landolph, J., Peterson, H. and Heidelberger, C. (1978) Nature 276, 508-510.
21. Ashman, C. and Davidson, R. (1981) Molec. Cell Biol. 1, 254-260.