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SHORT COMMUNICATION

Enhancement of Topoisomerase I-Mediated Unwinding of Supercoiled DNA by the Radioprotector WR-33278

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HOLWITT, E. A., KODA, E., AND SWENBERG, C. E. Enhancement of Topoisomerase I-Mediated Unwinding of Supercoiled DNA by the Radioprotector WR-33278. *Radiat. Res.* 124, 107–109 (1990).

The radioprotector WR-33278, the disulfide of WR-1065 (*N*-(2-mercaptoethyl)-1,3-diaminopropane), is shown to stimulate eukaryotic topoisomerase I unwinding of negatively supercoiled DNA. This observation suggests the possibility that some protection may be conferred to DNA either by a decrease in its supercoiled state or by altering directly other enzymatic processes. This is the first report of a radioprotective compound stimulating an enzyme involved in DNA structure and synthesis.

INTRODUCTION

Cellular DNA is one of the critical targets for ionizing radiation. To mitigate the effects of ionizing radiation, the U.S. Army Medical Research and Development Command has synthesized several radioprotective drugs, primarily aminothiol compounds. Many mechanisms responsible for their radioprotective action have been proposed, including radical scavenging (*1*), hydrogen atom donation to DNA carbon center radicals (*2*), enhancement of DNA repair processes (*3*), and reduction in the target volume. All of these processes require that the radioprotector or its metabolite be located within molecular distances, less than 50Å, from DNA.

Recent experimental studies of the radioprotector WR-1065 (*N*-(2-mercaptoethyl)-1,3-diaminopropane), the dephosphorylated product of WR-2721 (*S*-2-(3-aminopropylamino)ethylphosphorothioic acid), have shown that it binds with DNA at physiological pH (*4, 5*). However, the rapid rate of WR-1065 autooxidation (6) and its strong dependence on trace metal ions strongly suggest that locally the DNA neighborhood contains not only the chemical WR-1065, but also its symmetric disulfide, WR-33278. Prutz (*7*) has recently shown that disulfide radicals, which WR-33278 can form, may be the active form of sulfhydryl radioprotectors. For these reasons we restricted our studies to WR-33278. Furthermore, Holwitt and co-workers (unpublished) have shown that the binding of WR-33278 to calf thymus DNA is cooperative and dependent on salt concentration. WR-33278 binds to DNA with an association constant similar to that reported for WR-1065 (*4*). We expect that the experimental results for WR-1065 will be similar. The study was conducted to determine whether WR-33278 alters eukaryotic topoisomerase I unwinding of supercoiled DNA. WR-33278 was chosen because of the chemical similarity of the polyamine spermidine and WR-33278, the observation that both bind to DNA backbone primarily through electrostatic interactions, and the recently reported observation by Srivenugopal and Morris that calf thymus topoisomerase I unwinding of supercoiled DNA was stimulated by spermidine (*8*).

We first present the experimental protocol, followed by the main experimental results (reported in Figs. 1 and 2). Our results indicate that WR-33278 enhances topoisomerase I unwinding of supercoiled DNA. To our knowledge, this constitutes the first observation of an enzyme process enhanced by a radioprotective agent. The paper concludes with a discussion of the superhelical state of prokaryotic and eukaryotic DNA and possible implications for radioprotector–enzyme interaction.

METHODS

Plasmid pIBI30 was isolated by the alkaline lysis method from *Escherichia coli* (*9*). Calf thymus Type I DNA topoisomerase was purchased from Bethesda Research Laboratory and was used as received. One unit of activity was defined as the amount of enzyme needed to relax 1 μg of supercoiled DNA in 1 h. The radioprotector WR-33278 was obtained from Walter Reed Medical Research Institute and was used as received. Topoisomerase reactions were performed as described by Srivenugopal *et al.* (*10*). Stock solutions were as follows: 5× KCl (100 m*M*); spermidine (16 ×

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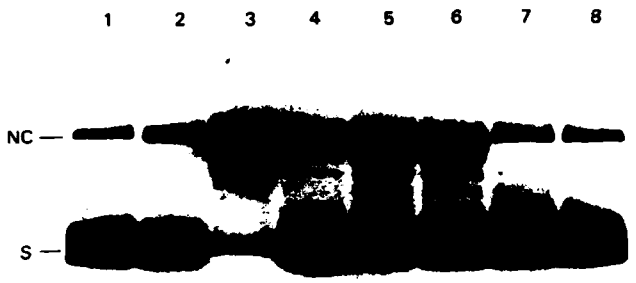

FIG. 1. Photograph of agarose gel illustrating the stimulation of topoisomerase I unwinding of supercoiled DNA by spermidine and WR-33278. S denotes supercoiled state; NC denotes the nicked circular form and/or the completely relaxed form of DNA. Numbers in parentheses are final concentrations for each gel lane. Controls: Lane 1, pIBI30 DNA alone (33.3 μg/ml); Lane 2, pIBI30 DNA (33.3 μg/ml) and topoisomerase (13.3 units/ml); Lane 3, DNA, topoisomerase, and spermidine (1.65 mM); Lanes 4 to 8, DNA, topoisomerase, and varying concentrations of WR-33278 (50, 100, 200, 500, and 1000 μM, respectively).

mM), whose pH adjusted to 7.5 with the basic form of Tris; and WR-33278, which required no pH adjustment. pIBI30 DNA (125 μg/ml) and topoisomerase (0.0667 units/μl) were prepared in reaction buffer: 20 mM Tris (pH 7.5), 0.5 mM DTT, and 6% glycerol. Topoisomerase was assayed by incubating 1 μg (0.101 mM in bases) of plasmid DNA with 0.4 units of enzyme in 20 mM of KCl. This salt concentration is below the optimum salt concentration of approximately 180 mM KCl needed for maximum topoisomerase activity (*11, 12*). It was chosen to enhance the demonstration of any stimulatory effect by polyamines and WR-33278 of topoisomerase I activity. Total sample volume for each tube was 30 μl. Reactions were carried out at 37°C and terminated after 3 h by addition of a Sarkosyl/EDTA mixture to a final concentration of 1% and 20 mM, respectively. Conversion of supercoiled DNA to relaxed forms of DNA was determined by gel electrophoresis (1.3% agarose). DNA was subjected to electrophoresis at 60 V in Tris acetate EDTA buffer until the tracking dye (bromphenol blue) was approximately 1 cm from the leading edge; this usually took 3 h. Gels were stained with ethidium bromide.

RESULTS

Figure 1 is a photograph of representative DNA topoisomerase I assay on agarose gel. The relaxed or open circle form of the plasmid is at the top of the gel, and the supercoiled form is at the bottom. When topoisomerase I relaxes DNA, the lower band's density decreases. Lane 1 is pIBI30 alone and shows where the relaxed and supercoiled forms of the plasmid run under our electrophoresis conditions. Under the assay conditions employed (20 mM KCl), topoisomerase I has low activity, and lane 2 of Fig. 1 demonstrates this lack of supercoil unwinding by the enzyme during the time allowed for the enzyme assay. Lane 3 of Fig. 1 shows clearly the stimulation of calf thymus type I topoisomerase by spermidine as previously reported by Srivenugopal and Morris (*8*) for native ColE1 DNA. It can be seen in Lanes 4–6 in Fig. 1 that WR-33278 also stimulates the topoisomerase I unwinding of supercoiled DNA and produces a ladder of isomers similar to that produced in the presence of spermidine. Also evident from these lanes is that an increase in the concentration of WR-33278 increases the amount of stimulation. A similar result was observed for spermidine-enhanced stimulation of topoisomerase I (*8*). Lanes 7 and 8 in Fig. 1 show a pattern similar to Lane 1. For these lanes the molar ratios of WR-33278 to DNA bases was above 2:1, and we have demonstrated using spectrophotometry that for these ratios, DNA precipitates; hence the lack of a ladder as shown in Lanes 3 through 6. The DNA appears on the gel because it is resolubilized by the detergent added to stop the reaction. The data in Fig. 2 demonstrate that neither spermidine (Lane 3) nor WR-33278 (Lanes 6–8) in the absence of topoisomerase I relaxes DNA.

DISCUSSION

The superhelical state of intracellular DNA is an important structural determinant of the function of DNA in cells (*13–15*). Superhelicity of DNA is controlled by a class of enzymes called topoisomerases, which are ubiquitous both in prokaryotic and in eukaryotic cells. These enzymes alter the topological conformation of DNA by nicking and resealing the DNA sugar–phosphate backbone. Those that change the DNA linking number by unity are called type I enzymes; type II enzymes change the link number by two. Type I enzymes act by producing transient single-strand breaks in DNA, whereas type II topoisomerases introduce transient double-stranded breaks (*16–18*).

Our experimental observation of the stimulation of calf thymus topoisomerase I action by WR-33278 is similar to

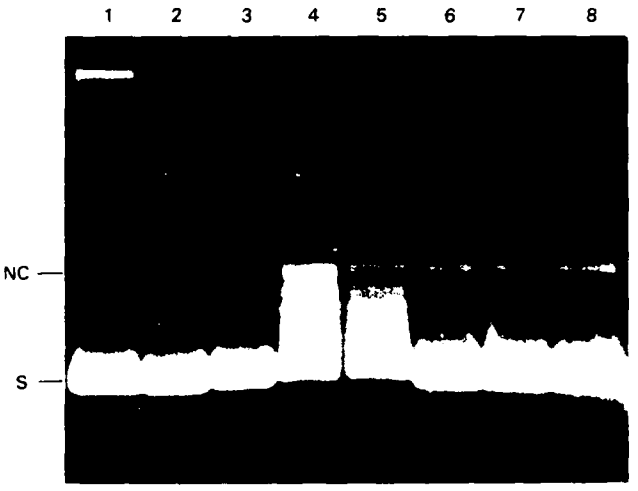

FIG. 2. Photograph of agarose gel demonstrating lack of supercoil unwinding in absence of topoisomerase I. S denotes supercoiled state; NC denotes the nicked circular form and/or the completely relaxed form of DNA. Numbers in parentheses are final concentrations for each gel lane. Lanes 1 and 2, same as in Fig. 1; Lane 3, DNA (33.3 μg/ml) and spermidine (1.65 mM); Lanes 4 and 5, DNA and topoisomerase (13.33 units/ml) with 1.65 and 2.75 mM spermidine. Lanes 6 to 8, DNA and WR-33278 (50, 100, and 200 μM, respectively).

the enhanced relaxation of supercoils by spermidine reported by Srivenugopal and Morris (*8*). This is a reasonable result in view of the similarity in their structure since WR-33278 may loosely be considered a polyamine containing a disulfide bond. In addition we note that both WR-33278 and spermidine (*19, 20*) bind externally to DNA through electrostatic interactions with the charged phosphate oxygen anions (*21*). Because supercoiled domains exist in both prokaryotic and eukaryotic cells, our observation suggests that radioprotectants may confer some protection to the genome by decreasing the supercoiling of DNA. The decrease in superhelicity could produce a decrease in the initial damage incurred and/or change the functional properties of DNA. If the first process is operative, then the critical DNA damage sites or "hot spots" would correspond to those DNA regions where the superhelicity is large. The second mechanism suggests possible changes in metabolic processes, a virtually unexplored field, although WR-1065 has been reported to enhance DNA repair (*22, 23*).

The molecular mechanism responsible for the stimulation of eukaryotic type I topoisomerase by WR-33278 is unclear. Both compounds bind to DNA but whether a transient tertiary complex is formed is not known and has not been investigated. The binding of WR-33278, even at a DNA site remote from the topoisomerase site of action, may provide a mechanism for stimulation of topoisomerase I action by conferring enhanced stabilization to the DNA backbone. These possibilities are currently under study. Nevertheless, the observation that WR-33278 stimulates topoisomerase I unwinding of the supercoiled state suggests new mechanisms by which radioprotective chemicals induce protection against ionizing radiation.

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REFERENCES

1. T. L. PHILLIPS, Rationale for initial clinical trials and future development of radioprotectants. *Cancer Clin. Trials* **3**, 165–173 (1980).
2. R. E. DURAND, Radioprotection by WR-2721 *in vitro* at low oxygen tensions. Implications for its mechanism of action. *Br. J. Cancer* **47**, 387–392 (1983).
3. E. RIKLIS, R. KOB, M. GREEN, A. PRAGER, R. MARKO, and M. MINTSBERG, Increased radioprotection attained by DNA repair enhancement. *Pharmacol. Ther.* **39**, 311–322 (1988).
4. G. D. SMOLUK, R. C. FAHEY, and J. F. WARD, Interaction of glutathione and other low-molecular-weight thiols with DNA: Evidence for counterion condensation and coion depletion near DNA. *Radiat. Res.* **114**, 3–10 (1988).
5. S. ZHENG, G. L. NEWTON, G. GONICK, R. C. FAHEY, and J. F. WARD, Radioprotection of DNA by thiols: Relationship between the net charge on a thiol and its ability to protect DNA. *Radiat. Res.* **114**, 11–27 (1988).
6. J. E. BIAGLOW, R. W. ISSELS, L. E. GERWECK, M. E. VARNES, B. JACOBSON, J. B. MITCHELL, and A. RUSSO, Factors influencing the oxidation of cysteamine and other thiols: Implications for hyperthermic sensitization and radioprotection. *Radiat. Res.* **100**, 298–312 (1984).
7. W. A. PRUTZ, Chemical repair in irradiated DNA solutions containing thiols and/or disulphides. Further evidence for disulphide radical anions acting as electron donors. *Int. J. Radiat. Biol.* **56**, 21–33 (1989).
8. K. S. SRIVENUGOPAL and D. R. MORRIS, Differential modulation by spermidine of reactions catalyzed by type I prokaryotic and eukaryotic topoisomerases. *Biochemistry* **24**, 4766–4771 (1985).
9. F. M. AUSUBEL, R. BRENT, R. E. KINGSTON, D. D. MOORE, J. G. SEIDMAN, J. A. SMITH, and K. STRAHL (Eds.), *Current Protocols in Molecular Biology*, pp. 1.71–1.74. Wiley–Interscience, New York, 1989.
10. K. S. SRIVENUGOPAL, D. E. WEMMER, and D. R. MORRIS, Aggregation of DNA by analogs of spermidine: enzymatic and structural studies. *Nucleic Acids Res.* **15**, 2563–2580 (1987).
11. B. L. MCCONAUGHY, L. S. YOUNG, and J. J. CHAMPOUX, The effect of salt on the binding of the eukaryotic DNA nicking-closing enzyme to DNA and chromatin. *Biochim. Biophys. Acta* **655**, 1–8 (1981).
12. J. J. CHAMPOUX, Evidence for an intermediate with a single-strand break in the reaction catalyzed by the DNA untwisting enzyme. *Proc. Natl. Acad. Sci. USA* **73**, 3488–3491 (1976).
13. M. GELLERT, DNA topoisomerase. *Annu. Rev. Biochem.* **50**, 879–910 (1981).
14. K. DRLICA, Biology of bacterial deoxyribonucleic acid topoisomerase. *Microbiol. Rev.* **48**, 273–289 (1984).
15. J. C. WANG, DNA topoisomerases. *Annu. Rev. Biochem.* **54**, 665–697 (1985).
16. F. B. FULLER, Decomposition of linking number of a closed ribbon: A problem from molecular biology. *Proc. Natl. Acad. Sci. USA* **75**, 3557–3561 (1978).
17. P. O. BROWN and N. R. COZZARELLI, A sign inversion mechanism for enzymatic supercoiling of DNA. *Science* **206**, 1081–1083 (1979).
18. K. MIZUUCHI, L. M. FISHER, M. H. O'DEA, and M. GELLERT, DNA gyrase action involves introduction of transient double-stranded breaks into DNA. *Proc. Natl. Acad. Sci. USA* **77**, 1847–1851 (1980).
19. B. C. HOOPES and W. R. MCCLURE, Studies on the selectivity of DNA precipitation by spermine. *Nucleic Acids Res.* **20**, 5413–5422 (1981).
20. I. BAEZA, P. GARIGLIO, L. M. RANGEL, P. CHAVEZ, L. CERVANTES, C. ARGUELLO, J. E. MORGAN, J. W. BLANKENSHIP, and H. R. MATTHEWS, Electron microscopy and biochemical properties of polyamine-compacted DNA. *Biochemistry* **26**, 6387–6392 (1987).
21. M. J. MARTON and D. R. MORRIS, In *Inhibition of Polyamine Metabolism: Biological Significance and Basis for New Therapy*, (P. P. McCann, A. E. Pegg, and A. Sjoerdma, Eds.), pp. 79–105. Academic Press, Orlando, 1987.
22. E. LOWENSTEIN, J. L. GLEESON, E. HECHT, R. FACTOR, C. GOLDFISCHER, A. CAJIGAS, and J. J. STEINBERG, Excision repair is enhanced by WR2721 radioprotection. In *Terrestrial Space Radiation and Its Biological Effects* (P. D. McCormack, C. E. Swenberg, and H. Bucker, Eds), pp. 697–714. Plenum, New York, 1989.
23. C. E. SWENBERG, DNA and radioprotection. In *Terrestrial Space Radiation and Its Biological Effects* (P. D. McCormack, C. E. Swenberg, and H. Bucker, Eds), pp. 675–695. Plenum Press, New York, 1989.