Noguchi, H., Veer Reddy, G. P., & Pardee, A. B. (1983) *Cell (Cambridge, Mass.) 32*, 443–451.
Paoletti, C., Le Pecq, J. B., Dat-Xuong, N., Juret, P., Garnier, H., Amiel, J. L., & Rouesse, J. (1980) in *Recent Results in Cancer Research* (Mathé, G., & Muggia, F. M., Eds.) pp 107–123, Springer-Verlag, Heidelberg.
Pelaprat, D., Oberlin, R., Le Guen, I., Roques, B. P., & Le Pecq, J. B. (1980a) *J. Med. Chem. 23*, 1330–1335.
Pelaprat, D., Delbarre, A., Le Guen, I., Roques, B. P., & Le Pecq, J. B. (1980b) *J. Med. Chem. 23*, 1336–1343.
Peters, W. (1984) *Handb. Exp. Pharmacol. 68*, 423–445.
Pommier, Y., Mattern, M. R., Schwartz, R. E., Zwelling, L. A., & Kohn, K. W. (1984a) *Biochemistry 23*, 2927–2932.
Pommier, Y., Schwartz, R. E., Kohn, K. W., & Zwelling, L. A. (1984b) *Biochemistry 23*, 3194–3201.
Riou, G. F., & Gutteridge, W. E. (1978) *Biochimie 60*, 365–379.
Riou, G., Gabillot, M., Douc-Rasy, S., Kayser, A., & Barrois, M. (1983) *Eur. J. Biochem. 134*, 479–484.
Riou, G., Gabillot, M., Barrois, M., Breitburd, D., & Orth, G. (1985) *Eur. J. Biochem. 146*, 483–488.
Roques, B. P., Pelaprat, D., Le Guen, I., Porcher, G., Gosse, C., & Le Pecq, J. B. (1979) *Biochem. Pharmacol. 28*, 1811–1815.
Ryoji, M., & Worcel, A. (1984) *Cell (Cambridge, Mass.) 37*, 33–41.
Sander, M., & Hsieh, T. S. (1983) *J. Biol. Chem. 258*, 8421–8428.
Siegel, L. M., & Monty, K. J. (1966) *Biochim. Biophys. Acta 112*, 346–362.
Tewey, K. M., Chen, G. L., Nelson, E. M., & Liu, L. F. (1984) *J. Biol. Chem. 259*, 9182–9187.
Wang, J. C. (1985) *Annu. Rev. Biochem. 54*, 665–697.

Formation of Methyl Ester of 2-Methylglyceric Acid from Thymine Glycol Residues: A Convenient New Method for Determining Radiation Damage to DNA†

Karl A. Schellenberg*,‡ and James Shaeffer§

Department of Biochemistry and Department of Radiation Oncology and Biophysics, Eastern Virginia Medical School, Norfolk, Virginia 23501

Received August 13, 1985

ABSTRACT: Thymine glycol residues in DNA or thymidine were converted to methyl 2-methylglycerate by reaction with alkaline borohydride followed by methanolic HCl. The product was labeled either from [^{3}H]DNA or from [^{3}H]borohydride and was followed by cochromatography with authentic ^{14}C-labeled material. Following acid hydrolysis, the identity of 2-methylglyceric acid was confirmed by high-resolution mass spectrometry, NMR, IR, and elemental analysis. Treatment of DNA or thymidine with X-irradiation, with H_2O_2 and Fe^{2+}, with H_2O_2, Cu^{2+}, and ascorbate, and with H_2O_2 and ultraviolet light, permanganate, or sonication all produced methyl 2-methylglycerate in varying amounts after alkaline borohydride and methanolic HCl, whereas untreated DNA did not. The data indicate that certain oxidants including hydroxyl radicals generated by chemical means or from radiolysis of water convert thymine residues to thymine glycols in DNA, which can be determined as methyl 2-methylglycerate.

The oxidation of thymine in DNA to thymine glycol represents a major chemical change caused by the action of ionizing radiation (Hariharan & Cerutti, 1972; Cerutti, 1976), sonication (McKee et al., 1977; Dooley et al., 1984), permanganate (Rubin & Schmid, 1980; Frenkel et al., 1981), Fenton's reagent (Floyd, 1981; Schellenberg et al., 1981), and ultraviolet light and hydrogen peroxide (Demple & Linn, 1982). The active systems, except for permanganate, all have in common the generation of hydroxyl radicals, which in addition to attacking thymines cause DNA strand breaks (Ward & Kuo, 1976; Lesko et al., 1980; Brawn & Fridovich, 1981) and cross-links (Lesko et al., 1982). Oxidation of thymine, presumably to the glycol, can lead to mutation in the Ames TA 102 *Salmonella* strain (Levin et al., 1982), and the necessity for glycol removal is implied by the presence of a specific endonuclease (Friedberg et al., 1981; Demple & Linn, 1982). The thymine glycol moiety may also be directly mutagenic (Wang et al., 1979). Glycol residues in DNA have been inferred from the formation of neutral species that contained the methyl carbon on treatment with alkali (Hariharan & Cerutti, 1972; Hariharan, 1980), and recently, the modified residues have been unequivocally identified as thymine glycol, 5,6-dihydroxy-5,6-dihydrothymine, by enzymatic hydrolysis of modified DNA to nucleosides and comparison with known thymidine glycol (Frenkel et al., 1981; Teebor et al., 1982). Other methods of thymine glycol estimation include immunoassay (Wallace, 1983; Rajagopalan et al., 1984) and direct determination in urine by chromatography (Cathcart et al., 1984). Earlier studies have shown that hydroxyl radical generating systems converted thymidine to thymidine glycol, which could be detected as 2-methylglyceric acid on reduction with borohydride and hydrolysis (Schellenberg, 1979; Schellenberg et al., 1981). We report here a convenient modification of the reaction allowing quantitative and specific determination of thymine glycol formation in DNA as methyl 2-methylglycerate.

† This work was supported by the Eastern Virginia Medical Authority.
‡ Department of Biochemistry.
§ Department of Radiation Oncology and Biophysics.

0006-2960/86/0425-1479$01.50/0

Experimental Procedures

Materials. Radiochemicals were obtained from Amersham-Searle; purity and identity of labeled thymidines were determined by thin-layer chromatography. Sodium borohydride was assayed by titration (Lyttle et al., 1952), and [^{3}H]borohydride was assayed by reduction of 4-carboxybenzaldehyde with separation of labeled 4-carboxybenzyl alcohol by TLC.[1] Inorganic chemicals were reagent-grade, and other chemicals were from Sigma. CHO cells were obtained from Dr. J. J. Starling and were grown in McCoy's 5A medium with 10% fetal calf serum and 0.05 mg/mL gentamycin, in an incubator at 37 °C with 5% CO_2 in the gas phase.

Physical Treatments. X-irradiation was carried out as previously described (Schellenberg et al., 1981). Sonication utilized the Bronwill Biosonik IV with a 1.5 cm diameter probe immersed in the solution contained in a 20-mL beaker surrounded by an ice bath. Power was determined by calorimetry. The Ultra-Violet Products, Inc., Model PCQ9G-1 2.5 quartz immersion lamp with 80–90% energy in the 254-nm band was used for ultraviolet photolysis. The lamp was partially immersed in the solution contained in a 15-mL centrifuge tube surrounded by an ice bath. The system was calibrated by ferrioxalate actinometry (Bowman & Damas, 1976).

PC and TLC. Bakerflex SG-1B silica gel (SG), Whatman 3 mm (P), or Brinkmann MN cellulose (CE) sheets were used, with the systems numbered as follows: (1) SG, ethyl acetate; (2) SG, ethyl acetate–methanol, 9:1; (3) CE, 1-butanol–acetic acid–water, 12:3:5; (4) P, ethyl acetate–2-propanol–ammonium hydroxide, 5:3:2.

[^{3}H]DNA. Approximately 4 × 10^6 CHO cells were plated in a 175-cm flask with 30 mL of medium containing 0.01 mM thymidine and 0.1 mCi of [*methyl*-^{3}H]thymidine and allowed to grow to confluence in 3 days. Cells were trypsinized, washed with PBS, and suspended in 0.1 M NaCl–0.1 M Na_3EDTA, pH 8. DNA was isolated (Marmur, 1961), and the material was dissolved in 0.1 M NaCl and 0.02 M Na_2HPO_4. Nonlabeled DNA solutions were prepared as previously described (Schellenberg et al., 1981).

2-Methylglyceric Acid. [*methyl*-^{14}C]Thymidine was converted to thymidine glycol (Iida et al., 1971), treated with excess 0.5 M NaOH and 0.13 M $NaBH_4$ at 25 °C for 2 h, and hydrolyzed with 3 M HCl at 105 °C for 8 h. The product was isolated by ion exchange chromatography on Bio-Rad AG 1-X10 with a gradient of water to 1 M ammonium acetate. The acid eluted at about 0.3 M in over 95% yield from thymidine glycol. After lyophilization, the material was a colorless oil. Anal. Calcd for $C_4H_8O_4 \cdot H_2O$: C, 34.78; H, 7.30. Found: C, 34.72; H, 7.77. NMR (D_2O) showed the following: δ 1.34 (s, 3, CH_3), 3.52 (d, 1, J = 11.8 Hz, Ha), and 3.78 (d, 1, J = 11.8 Hz, Hb). Ha and Hb are on the chiral methylene group giving the splitting. High-resolution MS gave m/e 121.0509 ± 0.002 (calcd 121.0498 for MH^+ of $C_4H_8O_4$). TLC with system 3 had R_f 0.70.

Methyl 2-Methylglycerate (MMG). Prepared by esterification of 2-methylglyceric acid with methanol–HCl at 60 °C for 1 h. The yield was quantitative. The ester was volatile, partly extractable from buffered aqueous solution with ethyl acetate, and VPC with a carbowax column at 55 °C gave a retention time of 1.32 compared with that of *n*-butyl acetate = 1.00. TLC with system 1 had R_f 0.44; TLC with system 2 had R_f 0.65; PC with system 4 had R_f 1.

Determination of MMG in DNA with NaB^{3}H$_4$. Nonlabeled calf thymus DNA 0.5 mM in bases, in 0.1 M NaCl–20 mM Na_2HPO_4 in a volume of 5 mL, after treatment to produce glycol residues, was lyophilized. One milliliter of 0.1 M NaOH and 5 mM NaB^3H_4, 7 × 10^{10} dpm/mmol, was added and allowed to react 1 h at 37 °C. The mixture was acidified with 2.5 mL of 6 M HCl, lyophilized, and treated with 0.5 mL of 1.4 M HCl in dry methanol 1 h at 60 °C. The ester was determined by PC with system 4, eluting the material with R_f 0.9–1, and TLC with system 1. Following determination of peaks with a Vanguard strip scanner, radioactivity was determined with a Beckman LS100 scintillation counter by cutting the TLC strip into segments, treating it with 0.3 mL of 0.2 M NaOH at 90 °C for 30 min, acidifying it with 1 drop of glacial acetic acid and addition of 2 mL of ACS (Amersham) scintillation fluid. Confirmation was obtained by addition of [*methyl*-^{14}C]thymidine to the DNA before reaction or [^{14}C]MMG to the solution prior to TLC and measurement of the ^{3}H/^{14}C ratio throughout the ester peak. In all cases where MMG was formed, the ^{3}H/^{14}C ratio was constant. Internal ^{3}H and ^{14}C standards were included, and dpm in dual-labeled samples were calculated with a computer algorithm. Identity in representative experiments was further confirmed by hydrolysis in 3 M HCl for 1 h at 90 °C and cochromatography with authentic 2-methylglyceric acid on the ion-exchange column and TLC with system 3.

Determination of MMG in [^{3}H]DNA. The procedure was similar to the above on a smaller scale, with [^{3}H]DNA from CHO cells as a source of ^{3}H label. The material was usually denatured by heating at 100 °C for 15 min; it was treated to form glycol residues, and then 0.1 mL was reacted with 0.02 mL of 0.5 M NaOH and unlabeled 0.12 M $NaBH_4$ at 37 °C for 1 h, followed by acidification with 0.02 mL of 5 M acetic acid, lyophilization, esterification with 0.2 mL of dry methanolic 1.4 M HCl at 60 °C for 1 h, TLC with system 1, and confirmation with [^{14}C]thymidine as described above.

Table I: MMG from DNA[a]

^{3}H label	treatment	MMG (mol/1000 bases)
$NaBH_4$	none	<0.008
$NaBH_4$	X-irradiation, 60 krad	3.2
$NaBH_4$	H_2O_2, 10 mM; Fe^{2+}, 20 mM	2.4
$NaBH_4$	H_2O_2, 0.2 M; UV, 0.74 W for 30 min	28
$NaBH_4$	H_2O_2, 30 mM; ascorbate, 3 mM; Cu^{2+}, 3 mM	3.7
$NaBH_4$	sonication, 33 W for 30 min	0.11
DNA	none	0.06
DNA	HCl, 0.2 M, 50 °C for 30 min	0.05
DNA	H_2O_2, 10 mM; Fe^{2+}, 20 mM	9.4
DNA	Fe^{2+}, 20 mM	2.6
DNA	H_2O_2, 30 mM	0.26
DNA	H_2O_2, 30 mM; ascorbate, 3 mM; Cu^{2+}, 3 mM	15.2
DNA	H_2O_2, 30 mM; ascorbate, 3 mM	7.8
DNA	H_2O_2, 30 mM; Cu^{2+}, 3 mM	1.8
DNA	ascorbate, 3 mM; Cu^{2+}, 3 mM	4.6
DNA	$KMnO_4$, 10 mM	22
DNA	$KMnO_4$, 50 mM	78
DNA	DNA not heated; $KMnO_4$, 10 mM	2.8

[a] DNA treated for 15 min at 0 °C unless noted otherwise and then assayed for MMG as described in the text. MMG labeled with ^{3}H from either NaB^3H_4 or [^{3}H]DNA.

Results and Discussion

Treatment of DNA solutions with various hydroxyl radical generating systems produced MMG, as shown in Table I.

[1] Abbreviations: CHO, Chinese hamster ovary; EDTA, ethylenediaminetetraacetic acid; MMG, methyl 2-methylglycerate; IR, infrared; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; PC, paper chromatography; UV, ultraviolet; SDS, sodium dodecyl sulfate; TLC, thin-layer chromatography; VPC, vapor-phase chromatography; MS, mass spectrometry.

Table II: MMG from Thymidine[a]

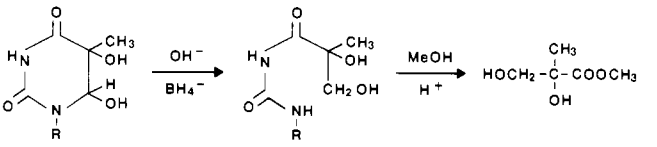
treatment	MMG (% yield)
H_2O_2, 5 mM; Fe^{2+}, 10 mM	19
H_2O_2, 20 mM; Fe^{2+}, 40 mM	33
H_2O_2, 20 mM; Ti^{3+}, 80 mM	12
H_2O_2, 20 mM; ascorbate, 10 mM; Cu^{2+}, 10 mM	22
H_2O_2, ascorbate, and Cu^{2+} for 5 min, then add thymidine	0
UV	0
H_2O_2, 0.1 M; UV	2
H_2O_2, 0.3 M; UV	4
H_2O_2, 1 M; UV	9
sonication, 7 W in air	6
sonication, 16 W in air	17
sonication, 33 W in air	35
sonication, 33 W in nitrogen	0
sonication, 33 W in helium	0
$KMnO_4$, 10 mM	94

[a] [*methyl*-^{14}C]Thymidine, 5 mM, was treated as described in the table, with reactions for 5 min unless noted otherwise. UV was at 0.74 W for 10 min. Sonication was for 15 min with gas-phase bubbling through the mixture. After treatment, the mixture was processed as described for [^{3}H]DNA.

Thus, radiolysis of water, Fenton's reagent (Uri, 1952), UV photolysis of hydrogen peroxide (Baxendale & Wilson, 1957), sonication, and treatment with ascorbate, $CuSO_4$, and hydrogen peroxide (Vorhaben & Steele, 1967; Wong et al., 1974; Ishimitsu et al., 1977) all produced thymine glycol residues presumably via hydroxyl radical attack. The assay with tritium-labeled borohydride was particularly sensitive, with untreated DNA giving an extremely small quantity of ester, about 5 cpm above background under the conditions specified. The second series of experiments (Table I) utilizing [^{3}H]DNA gave a measurable value in untreated DNA, which may be due to radiolysis by the tritium which is known to give (hydroxymethyl)uracil as well as thymine glycol residues (Teebor et al., 1984). The material had been stored in the freezer for several weeks after preparation. Permanganate gave nearly quantitative yield of glycol from added thymidine and gave high yields of glycol and, hence, MMG from denatured, but not unheated, DNA in confirmation of previous work (Frenkel et al., 1981; Rubin & Schmid, 1980). DNA depurinated by acid treatment (Tamm et al., 1952) gave no MMG above that of the control. Table II summarizes experiments with thymidine in place of DNA. The Fenton-type reactions were all fast, and experiments (not shown) have indicated that premixing H_2O_2 and $FeSO_4$ for a few seconds before addition of thymidine gave no product. The formation of thymidine glycol by photolysis in the presence of H_2O_2 was dependent on large H_2O_2 concentrations, indicating that H_2O_2 was the species photolyzed. Sonication gave glycol in proportion to energy and required the presence of oxygen.

The procedure for MMG formation described here appeared to be satisfactory, since other experiments have indicated that the half-time for reduction of thymidine glycol by 1 M NaOH and 0.2 M $NaBH_4$ was 6 min at 23 °C and the half-time for hydrolysis of the reduced product from thymidine and esterification by 1.4 M HCl in methanol was 10 min at 23 °C. Variations in the DNA experiments indicated that the reactions had reached completion in the times given. TLC with system 3 of product from DNA that was labeled with NaB^3H_4 gave at least five peaks, and preliminary separation of the ester from more polar products by paper chromatography with system 4 was necessary to obtain uncontaminated ester peaks. TLC with system 1 of the thymidine reaction product showed only the peak of MMG, and TLC of [^{3}H]DNA product gave nearly all the ^{3}H at the origin and ester peaks, with an additional small peak with higher R_f than the ester on system 1.

Scheme I



We have shown that thymine glycol residues in DNA or thymidine glycol undergo the reactions shown in Scheme I. (Hydroperoxy)thymine glycols, such as 5-hydroxy-6-(hydroperoxy)dihydrothymine of 5-(hydroperoxy)-6-hydroxydihydrothymine residues, which are also formed from irradiation of thymine derivatives (Cadet & Teoule, 1972; Hahn & Wang, 1973), would probably undergo the same reactions, since borohydride is known to reduce organic hydroperoxides to alcohols (Teng et al., 1973; Beringer et al., 1972). We have not specifically tested the hydroperoxy compounds, however.

These reactions may be used as a specific probe for oxidative damage to DNA, such as that produced by ionizing radiation, sonication, or other agents that produce hydroxyl radicals. Adaptation of this method may be applicable to the in vivo determination of radiation damage, especially if 2-methylglyceric acid is found not to be a normal metabolite but is a degradation product of thymine glycol in vivo. The extreme reactivity and nonspecificity of hydroxyl radicals would be expected to lead to a mixture of *R* and *S* forms of the methylglyceric acid, although previous studies of the glycol isomers produced from irradiation of thymidine suggest that one isomer may predominate (Cadet & Teoule, 1972). Further studies are needed to clarify which isomers are present.

Experiments are presently under way and planned to extend this method to the determination of radiation damage to intact organisms by measurement of levels of 2-methylglyceric acid following irradiation and appropriate treatment and measurement of 2-methylglyceric acid that may be formed endogenously.

ACKNOWLEDGMENTS

Mass spectral determinations were carried out at the Middle Atlantic Mass Spectrometry Laboratory, a National Science Foundation Shared Instrumentation Facility. NMR studies were carried out with the assistance of Dr. William Bunnelle at the College of William & Mary Department of Chemistry and the Instrumentation Laboratory, Department of Chemical Sciences, Old Dominion University.

Registry No. Thymine glycol, 2943-56-8; methyl 2-methylglycerate, 19860-56-1; thymidine, 50-89-5; hydrogen peroxide, 7722-84-1; iron sulfate, 7720-78-7; potassium permanganate, 7722-64-7; copper sulfate, 7758-98-7; ascorbate, 10504-35-5; hydrochloric acid, 7647-01-0; hydroxyl, 3352-57-6.

REFERENCES

Baxendale, J. H., & Wilson, J. A. (1957) *Trans. Faraday Soc. 53*, 344–356.

Beringer, F. M., Ganis, P., Avitabile, G., & Jaffe, H. (1972) *J. Org. Chem. 37*, 879–886.

Bowman, W. D., & Damas, J. N. (1976) *J. Phys. Chem. 80*, 2434–2435.

Brawn, K., & Fridovich, I. (1981) *Arch. Biochem. Biophys. 206*, 414–419.

Cadet, J., & Teoule, R. (1972) *Tetrahedron Lett. 31*, 3225–3228.

Cathcart, R., Schwiers, E., Saul, R. L., & Ames, B. N. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 5633–5637.

Cerutti, P. A. (1976) in *Photochemistry and Photobiology of Nucleic Acids* (Wang, S. Y., Ed.) Vol. II, pp 375-401, Academic Press, New York.
Demple, B., & Linn, S. (1982) *Nucleic Acids Res. 10*, 3781-3789.
Dooley, D. A., Sack, P. G., & Miller, M. W. (1984) *Radiat. Res. 97*, 71-86.
Floyd, R. A. (1981) *Biochem. Biophys. Res. Commun. 99*, 1209-1215.
Frenkel, K., Goldstein, M. S., & Teebor, G. W. (1981) *Biochemistry 20*, 7566-7571.
Friedberg, E. C., Bonura, T., Love, J. D., McMillian, S., Radany, E. H., & Schultz, R. A. (1981) *J. Supramol. Struct. Cell. Biochem. 16*, 91-103.
Hahn, B. S., & Wang, S. Y. (1973) *Biochem. Biophys. Res. Commun. 54*, 1224-1232.
Hariharan, P. V. (1980) *Radiat. Res. 81*, 496-498.
Hariharan, P. V., & Cerutti, P. A. (1972) *J. Mol. Biol. 66*, 65-81.
Iida, S., & Hayatsu, H. (1971) *Biochim. Biophys. Acta 228*, 1-8.
Ishimitsu, S., Fujimoto, S., & O'Hara, A. (1977) *Chem. Pharm. Bull. 25*, 471-474.
Lesko, S. A., Lorentzen, R. J., & Ts'o, P. O. P. (1980) *Biochemistry 19*, 3023-3028.
Lesko, S. A., Drocourt, J. L., & Yang, S. U. (1982) *Biochemistry 21*, 5010-5015.
Levin, D. E., Hollstein, M., Christman, M. F., Schwiers, E. A., & Ames, B. N. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 7445-7449.
Lyttle, D. A., Jensen, E. H., & Struck, W. A. (1952) *Anal. Chem. 24*, 1843-1844.
Marmur, J. (1961) *J. Mol. Biol. 3*, 208-218.
McKee, J. R., Christman, C. L., O'Brien, W. D., Jr., & Wang, S. Y. (1977) *Biochemistry 16*, 4651-4654.
Rajagopalan, R., Melamede, R. J., Laspia, M. F., Erlanger, B. F., & Wallace, S. S. (1984) *Radiat. Res. 97*, 499-501.
Rubin, C. M., & Schmid, C. W. (1980) *Nucleic Acids Res. 8*, 4613-4619.
Schellenberg, K. A. (1979) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 38*, 501 (Abstr. 1433).
Schellenberg, K. A., Shaeffer, J., Nichols, R. K., & Gates, D. (1981) *Nucleic Acids Res. 9*, 3863-3872.
Tamm, C., Hodes, E., & Chargaff, E. (1952) *J. Biol. Chem. 195*, 49-63.
Teebor, G. W., Frenkel, K., & Goldstein, M. S. (1982) *Prog. Mutat. Res. 4*, 301-311.
Teebor, G. W., Frenkel, K., & Goldstein, M. S. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 318-321.
Teng, J. I., Kulig, M. J., Smith, L. L., Kan, G., & van Lier, J. E. (1973) *J. Org. Chem. 38*, 119-123.
Uri, N. (1952) *Chem. Rev. 50*, 375-454.
Vorhaben, J. E., & Steele, R. H. (1967) *Biochemistry 6*, 1404-1412.
Wallace, S. S. (1983) *Environ. Mutagen. 5*, 769-788.
Wang, S. Y., Hahn, B. S., Batzinger, R. P., & Beuding, E. (1979) *Biochem. Biophys. Res. Commun. 89*, 259-263.
Ward, J. F., & Kuo, I. (1976) *Radiat. Res. 66*, 485-498.
Wong, K., Morgan, A. R., & Paranchych, W. (1974) *Can. J. Biochem. 52*, 950-958.

Continuous Association of *Escherichia coli* Single-Stranded DNA Binding Protein with Stable Complexes of *rec*A Protein and Single-Stranded DNA†

Scott W. Morrical, Jongwon Lee, and Michael M. Cox*
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin—Madison, Madison, Wisconsin 53706
Received July 16, 1985

ABSTRACT: The single-stranded DNA binding protein of *Escherichia coli* (SSB) stimulates *rec*A protein promoted DNA strand exchange reactions by promoting and stabilizing the interaction between *rec*A protein and single-stranded DNA (ssDNA). Utilizing the intrinsic tryptophan fluorescence of SSB, an ATP-dependent interaction has been detected between SSB and *rec*A–ssDNA complexes. This interaction is continuous for periods exceeding 1 h under conditions that are optimal for DNA strand exchange. Our data suggest that this interaction does not involve significant displacement of *rec*A protein in the complex by SSB when ATP is present. The properties of this interaction are consistent with the properties of SSB-stabilized *rec*A–ssDNA complexes determined by other methods. The data are incompatible with models in which SSB is displaced after functioning transiently in the formation of *rec*A–ssDNA complexes. A continuous association of SSB with *rec*A–ssDNA complexes may therefore be an important feature of the mechanism by which SSB stimulates *rec*A protein promoted reactions.

The steps in homologous genetic recombination mediated by the *rec*A protein of *Escherichia coli* have been investigated through the study of DNA strand exchange reactions promoted by this enzyme in vitro. One of these in vitro systems has been especially informative. In the presence of ATP, the *rec*A protein promotes the exchange of complementary strands between homologous circular single-stranded and linear duplex DNA molecules derived from bacteriophage, forming nicked circular heteroduplex and linear single-stranded species as products. Strand exchange occurs in three distinguishable phases: (1) *presynapsis*, the cooperative binding of *rec*A protein to single-stranded DNA (ssDNA)[1] in the presence of

† This work was supported by National Institutes of Health Grant GM32335. S.W.M. was supported by Training Grant 5-T32 GM07215 from the National Institutes of Health.

0006-2960/86/0425-1482$01.50/0