INT. J. RADIAT. BIOL., VOL. 14, NO. 6, 567–571

Lack of *in vitro* repair of X-ray-induced chain breaks in DNA by the polynucleotide-joining enzyme

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(*Received* 14 *October* 1968)

The polynucleotide-joining enzyme does not repair (in one step) the single-strand chain breaks produced in DNA by x-irradiation in aqueous media. Preliminary data on the chemical nature of the ends of the broken strands suggest that nuclease action (to ' clean ' the termini), followed by polymerase action, is necessary before the irradiated DNA would become a substrate for the polynucleotide-joining enzyme.

1. Introduction

Internucleotide chain breaks in DNA induced by x-irradiation have been shown to be the primary lethal events in x-irradiated bacteria (Kaplan 1966, McGrath and Williams 1966). Several studies suggest that these chain breaks occur at the phosphodiester bond when DNA is irradiated in aqueous media (Collyns, Okada, Scholes, Weiss and Wheeler 1965, Scholes, Ward and Weiss 1960). Recently a ' polynucleotide-joining enzyme ' has been isolated from *E. coli* (Olivera and Lehman 1967) which catalyses the condensation of short polydeoxythymidylate chains (150 residues) to form deoxythymidylate polymers whose length has increased by as much as 20-fold. The enzyme requires a divalent cation (Mg^{++} or Ca^{++}), DPN, and polydeoxythymidylate chains with juxtaposed 5′-phosphoryl and 3′-hydroxyl termini (held in position by hydrogen bonding to large molecular weight polydeoxyadenylic acid). The juxtaposed polydeoxythymidylate chains are joined through the formation of 3′5′-phosphodiester linkages. A ' DNA-joining enzyme ' with similar requirements has also been isolated from *E. coli* and has been shown to repair pancreatic DNase produced single chain breaks in λ DNA (Zimmerman, Little, Oshinsky and Gellert 1967). A possibly identical enzyme has been isolated from extracts of *E. coli* K–12 by Gefter, Becker and Hurwitz (1967). An ATP-dependent enzyme system with related activity has been purified from *E. coli* infected with T4 bacteriophage (the ' ligase system ' of Weiss and Richardson 1967; also Gefter *et al.* 1967).

The availability of these specific joining enzymes now permits one to investigate the location(s) of the chain breaks produced by the x-irradiation of DNA. If the breaks occur such that juxtaposed 5′-phosphoryl and 3′-hydroxyl termini result, then the polynucleotide-joining enzyme of Olivera and Lehman (1967) should repair this damage and rejoin the chains. We have tested this hypothesis, and this paper presents data showing that chain breaks produced by the x-irradiation of DNA in aqueous solution are not directly reparable by the polynucleotide-joining enzyme.

2. Materials and methods

2.1. *Materials*

The polynucleotide-joining enzyme was kindly supplied by Dr. B. Olivera and Dr. I. R. Lehman, as was the substrate for the enzyme (a double-stranded homopolymer pair consisting of multiple 5′-^{32}P-dT units of about 150 residues, hydrogen-bonded to a poly dA chain of approximately 3000 residues). The buffer solution for the enzyme assays contained 1 ml. 1 M Tris-HCl pH 8, 0·5 ml. 0·2 M EDTA, 2·0 ml. 0·1 M $MgCl_2$, 0·5 ml. 10 mg/ml. bovine plasma albumin and was brought to 10 ml. with double-distilled water. A 0·5 mM solution of DPN was prepared and added separately to the incubation mixture. ^{14}C-thymine-labelled DNA was isolated from *E. coli* by the method of Smith (1962). Contaminating RNA was removed as described by Smith and Yoshikawa (1966). Before further dilution the extracted DNA had 72 800 c.p.m./0·1 ml. and 3·57 A_{260}/ml. A 1 : 5 dilution of DNA in 0·15 N NaCl was employed in the experiments described below.

2.2. *Irradiation of DNA and chain breakage*

Solutions of DNA, exposed to air, were irradiated with x-rays (50 kV, 50 mA; dose-rate 9·8 krads/min) for 2 min. An average of 15 single chain breaks per strand of DNA were produced under these conditions, whereas only 1·3 double chain breaks were produced. These estimates are based on determination of molecular weight of irradiated DNA by the use of 5–20 per cent alkaline (McGrath and Williams 1966) and neutral (Kaplan 1966) sucrose gradients.

2.3. *Action of polynucleotide-joining enzyme on substrate and irradiated DNA*

Preliminary experiments indicated that treatment of x-irradiated DNA with ' polynucleotide-joining enzyme ' did not result in an increase in the molecular weight of the single-strand pieces of DNA as assayed by subsequent denaturation in alkali and banding in alkaline sucrose gradients. To ensure that products produced by the irradiation were not toxic to the enzyme, it was deemed desirable to use x-irradiated ^{14}C-DNA and the ^{32}P-polythymidylic acid substrate of Olivera and Lehman (1967) in the same reaction mixture. These are the experiments presented here.

Three samples were prepared as summarized in the table. Samples 2 and 3 contained irradiated ^{14}C-DNA; samples 1 and 3 contained the polynucleotide-joining enzyme, and all samples contained the DPN co-factor, buffer concentrate, and ^{32}P-enzyme substrate. The enzyme was supplied in sufficient amount to enable repair of all the x-ray-induced single chain breaks (if they are of the proper specificity) as well as the joining of the ^{32}P-substrate. The DNA, enzyme, substrate mixtures were incubated for 30 min at 37°c. The enzyme action was terminated by the addition of 120 μl. of 0·05 M EDTA and the mixtures were brought to a pH $\sim$13 by the addition of NaOH. After a 10-min delay to permit alkaline-denaturation of the DNA, 0·2 ml. of each sample was layered on a 5–20 per cent alkaline sucrose gradient in 0·1 N NaOH and 0·01 M EDTA and centrifuged for 6·8 hours at 37 000 r.p.m. After centrifugation 5-drop fractions were collected and counted, following the procedure of Kaplan (1966). The scintillation counter was set for double-label counting, as described in the Nuclear Chicago manual. Data are plotted as radioactivity against fraction number.

Additives†	Samples		
	1 (no irradiation)	2 (19·6 krads)	3 (19·6 krads)
^{14}C-DNA	150‡	150	150
Buffer concentrate	30	30	30
DPN (0·5 mM)	5	5	5
5′-^{32}P-poly dT	60	60	60
Poly dA	5	5	5
Distilled water	—	70	—
Polynucleotide-joining enzyme (30 units/ml.)	70	—	70

† See § 2.
‡ Volumes in μl.

Assay for enzymic repair of x-irradiated DNA.

The first moment of each curve was used as a convenient index of sedimentation behaviour (Kaplan 1966), and ratios of the first moments were employed in the calculation of polynucleotide-joining enzyme activity.

3. Results

3.1. *Increase in molecular weight of dT polymers*

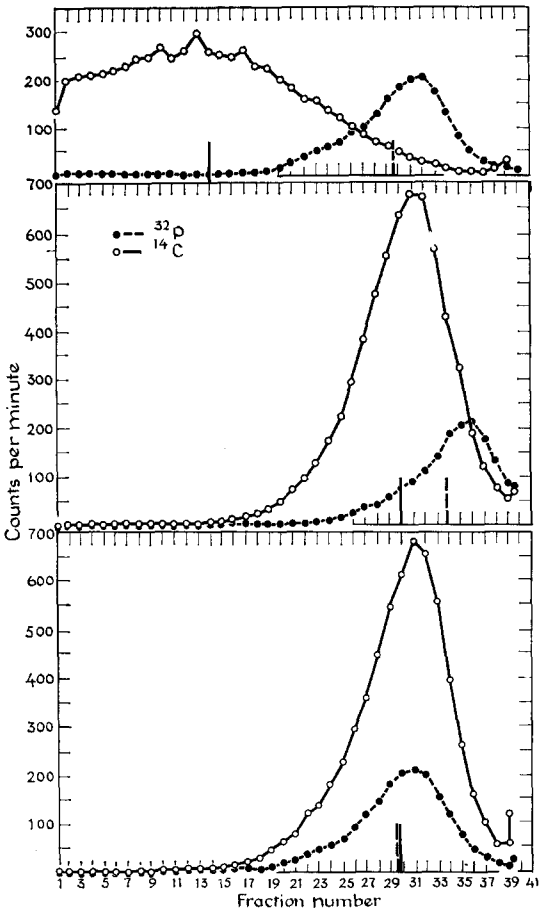
By comparison of the position of the enzyme-treated substrates (figure, top, bottom) with that of the control (figure, middle) it can be seen that the dT polymers have increased in molecular weight. To obtain a quantitative estimate of the increase in molecular weight, we have used the following relationship (Burgi and Hershey 1963):

$$\frac{D_2}{D_1} = \left(\frac{M_2}{M_1}\right)^a$$

where D is the distance sedimented and M is the molecular weight of the substrate with and without treatment with the polynucleotide-joining enzyme. The parameter a was taken as 0·40 for alkaline denatured DNA and ^{32}P–poly dT run in alkaline gradients, whereas a value of 0·35 was used for native DNA run in neutral gradients (Studier 1965). The D values were approximated by the use of normalized distance from the meniscus to the location of the first moment of each curve. A 3·7 and 3·9-fold increase in molecular weight of the ^{32}P-substrate is seen to occur in samples 1 and 3 respectively, after treatment with the polynucleotide-joining enzyme.

3.2. *Lack of rejoining of X-irradiated DNA*

Comparison of the positions of the sedimentation peaks and D values of the x-irradiated DNA (figure, middle) and the x-irradiated DNA incubated with polynucleotide-joining enzyme (figure, bottom) shows that no relevant change in molecular weight can be detected. Hence, within the limits of our experimental procedures, we can conclude that the chain breaks produced by the x-irradiation of DNA in aqueous solution are not directly reparable by the polynucleotide-joining enzyme.



Alkaline sucrose density gradient centrifugation of products of polynucleotide-joining enzyme. Samples (see table and § 2) were layered on sucrose gradients (5–20 per cent) containing 0·1 N NaOH and 0·01 M EDTA. Sedimentation was performed in a Spinco SW 39 rotor for 6·8 hours at 37 000 r.p.m. at room temperature. Top graph: unirradiated ^{14}C–DNA, ^{32}P–poly dT substrate and polynucleotide-joining enzyme. Middle graph: x-irradiated (19·6 krads) DNA, ^{32}P-substrate, no enzyme. Bottom graph: x-irradiated (19·6 krads) DNA, ^{32}P-substrate and enzyme. The short vertical bars under the peaks indicate the position of the first moments of the distribution of radioactivity. The direction of sedimentation is from right to left.

4. Discussion

These experiments have demonstrated that, in the same incubation mixture where short chains of poly dT are joined together, the polynucleotide-joining enzyme fails to repair single-strand chain breaks in DNA produced by x-irradiation. In view of the known specificity of this enzyme, we conclude that x-irradiation does not break polynucleotide chains at a phosphate bond such as to leave a 3′-hydroxyl group and a 5′-phosphate group in juxtaposition.

Current investigations in our laboratory on the chemical nature of the products resulting from chain breakage of calf-thymus DNA irradiated in aqueous media further support this conclusion. We observe that only about 33 per cent of the phosphate termini liberated by x-irradiation are 5′-phosphate termini.

We also observe significant damage to deoxyribose. Malonic aldehyde is produced (Krushinskaya and Shal'nov 1967) at a ratio of approximately 1 molecule per 3 single chain breaks. This ratio is not altered if the DNA is irradiated in a 0·001 M solution of histidine to help counteract the indirect effects of radiation (Freifelder 1965).

Although the polynucleotide-joining enzyme may well be required for the final step in the repair of chain breaks it appears that both nuclease action (to ' clean ' the termini) and polymerase action are first required.

ACKNOWLEDGMENTS

We are indebted to Dr. B. Olivera and Dr. I. R. Lehman for their helpful suggestions, kind assistance, and generous gifts of enzymes and substrates.

This investigation was supported by USPHS research grantCA–0 2896, research career development award CA–3709 (to K.C.S.) and Medical Scientist Fellowship (to D.S.K.) from the Life Insurance Medical Research Fund.

L'enzyme de jonction des polynucléotides ne répare pas en une seule étape les ruptures produites, sur une seule chaîne d'ADN, par des rayons x en milieu aqueux. Les premiers résultats concernant la nature chimique des terminaisons de ces chaînes après leur rupture, suggèrent que ces terminaisons doivent d'abord être nettoyées par l'action d'une nucléase suivie elle-même par l'action d'une polymérase, avant de permettre à l'ADN irradié de devenir un substrat pour l'action de l'enzyme de jonction.

Die Polynukleotid-Ligase schließt nicht (direkt) solche DNS-Einstrangbrüche, die durch Bestrahlung mit Röntgen-Strahlen in wässrigen Lösungen entstehen. Vorläufige Resultate von Untersuchungen über die chemische Struktur der Endgruppen dieser Brüche lassen darauf schließen, daß nacheinander ein nukleolytischer Abbau und ein DNS-Polymerase katalysierter Aufbau nötig sind als ' Reiningungschritte ', ehe die röntgen-bestrahlte DNS ein geeignetes Substrat für die Polynucleotid-Ligase-Reaktion ist.

REFERENCES

BURGI, E., and HERSHEY, A. D., 1963, *Biophys. J.*, **3**, 309.
COLLYNS, B., OKADA, S., SCHOLES, G., WEISS, J. J., and WHEELER, C. M., 1965, *Radiat. Res.*, **25**, 526.
FREIFELDER, D., 1965, *Proc. natn. Acad. Sci. U.S.A.*, **54**, 128.
GEFTER, M. L., BECKER, A., HURWITZ, J., 1967, *Proc. natn. Acad. Sci. U.S.A.*, **58**, 240.
KAPLAN, H. S., 1966, *Proc. natn. Acad. Sci. U.S.A.*, **55**, 1442.
KRUSHINSKAYA, N. P., and SHAL'NOV, M. I., 1967, *Radiobiology*, **7**, 36 (English translation by U.S.A.E.C.).
MCGRATH, R. A., and WILLIAMS, R. W., 1966, *Nature, Lond.*, **212**, 534.
OLIVERA, B., and LEHMAN, I. R., 1967, *Proc. natn. Acad. Sci. U.S.A.*, **57**, 1426.
SCHOLES, G., WARD, J. F., and WEISS, J., 1960, *J. molec. Biol.*, **2**, 379.
SMITH, K. C., 1962, *Biochem. biophys. Res. Commun.*, **8**, 157.
SMITH, K. C., and YOSHIKAWA, H., 1966, *Photochem. Photobiol.*, **5**, 777.
STUDIER, F. W., 1965, *J. molec. Biol.*, **11**, 373.
WEISS, B., and RICHARDSON, C. C., 1967, *Proc. natn. Acad. Sci. U.S.A.*, **57**, 1021.
ZIMMERMAN, S. B., LITTLE, J. W., OSHINSKY, C. K., and GELLERT, M., 1967, *Proc. natn. Acad. Sci. U.S.A.*, **57**, 1841.