The Effects of γ -Irradiation on the Priming by Deoxyribonucleohistone of Ribonucleic Acid Polymerase

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1. The effect of γ -irradiation of solutions of DNA and deoxyribonucleohistone (DNH) on their ability to prime the synthesis of RNA by DNA-dependent RNA polymerase has been studied. 2. The priming ability of both DNA and DNH decreased continuously with increasing radiation dose, but more rapidly with DNH. 3. These decreases have been compared with decreases in molecular weight and with the breakdown of the specific hydrogen-bonded structure of DNA. 4. It is concluded that a process was occurring during γ -irradiation of DNH that, although involving a decrease in molecular weight, did not diminish and even enhanced its priming ability. This is consistent with previous physicochemical evidence that γ -irradiation causes dissociation of histone from DNH.

Ionizing radiations impair the ability of DNA to prime the synthesis of RNA from nucleoside triphosphates in the presence of RNA polymerase (nucleoside triphosphate-RNA nucleotidyltransferase, EC 2.7.7.6) from *Escherichia coli* (Harrington, 1964; Zimmermann, Kröger, Hagen & Keck, 1964). This effect of γ -irradiation on DNA solutions has been attributed (Zimmermann *et al.* 1964) to the introduction of breaks in the polynucleotide chains, or destruction of bases, even at relatively low doses (5000 rads on 0.1% solutions, i.e. 0.17 eV/P atom, in the notation of Cox, Overend, Peacocke & Wilson, 1958). In the similar impairment of the priming ability of DNA caused by u.v. irradiation of DNA solutions, the denaturation of the DNA has also been implicated (Hagen, Keck, Kröger, Zimmermann & Lücking, 1965), as well as the other effects (especially destruction of thymine bases) mentioned above. A greater affinity of RNA polymerase for binding irradiated DNA has also been observed (Zimmermann, Kröger & Lücking, 1965).

When the soluble complex (DNH $\dagger$) of DNA with histone is irradiated with γ -rays (Peacocke & Preston, 1961; Lloyd & Peacocke, 1963*a*, 1966), the DNA, which forms the inner core of the complex, is degraded and denatured, though much less efficiently than when DNA is irradiated alone

(Peacocke & Preston, 1960*a,b*); in addition, the physicochemical evidence suggests that histone is dissociated from the DNA. It has been shown in several Laboratories (Barr & Butler, 1963; Hindley, 1963; Baxill & Philpot, 1963; Bonner & Huang, 1963; Huang, Bonner & Murray, 1964; Allfrey, Littau & Mirsky, 1963) that, when histone is attached to DNA, the ability of the DNA to prime RNA synthesis is greatly diminished. This has led to much experiment and speculation about the role of the histones in suppressing the genetic role of DNA. Because of this wider import, and because such studies might help to elucidate the effects of radiation on DNH itself, the effect of γ -irradiation in solution on the priming ability of DNH was examined. By choosing irradiation conditions that were the same as those employed in a previous physicochemical study (Lloyd & Peacocke, 1966) of the effect of γ -rays on the structure of DNH in solution, we also hoped that the effects of γ -rays on the priming ability might be correlated with the extent of degradation and denaturation that had been inferred from these earlier studies. While the present studies were in progress, our attention was drawn to the work of Weiss & Wheeler (1964), who reported that there was an initial decrease in the priming ability of DNH for *Micrococcus lysodeikticus* RNA polymerase after γ -irradiation with 12krads (on a solution 0.02% in DNA, i.e. 2.1 eV/P atom), but that with greater doses, up to 72 krad (i.e. 12.6 eV/P atom), the priming ability increased. This is consistent with the concept that irradiation tends to detach histone

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‡ Abbreviation: DNH, deoxyribonucleohistone.

from the DNA, which had also been inferred from the physicochemical studies (Peacocke & Preston, 1961; Lloyd & Peacocke, 1963a, 1966).

We now report studies on the effects of irradiation in the ability of DNA to prime the RNA polymerase of *E. coli*. Our observations are different from those of Weiss & Wheeler (1964), in that no increase in priming ability was observed at higher doses. However, a comparison of the loss of priming activity with the previously determined changes in molecular weight caused by irradiation allow the observations to be related to the degradation of the DNA and to the tendency of histone to dissociate from DNH during γ -irradiation.

MATERIALS AND METHODS

Isolation of polymerase. A 500 g. (wet wt.) sample of *E. coli* B was supplied by the Microbiological Research Establishment, Porton, Wilts. Cells were grown in continuous culture in a 17 l. working-volume culture vessel with a flow rate of 10.8 l./hr., giving a mean generation time of approx. 1 hr. at 37°. The culture medium contained: K_2HPO_4 (10.63 g.); KH_2PO_4 (5.31 g.); $(NH_4)_2SO_4$ (1.98 g.); $FeSO_4 \cdot 7H_2O$ (0.556 mg.); $MgCl_2 \cdot 6H_2O$ (203.3 mg.); glucose (8.0 g.) added as dextrosol (9.04 g.); tap water to 1 l., giving approx. pH 6.9. Cells (1.7 g. dry wt./l.) were harvested by centrifugation and washed with cold buffer (pH 7.9 at 5°) of the following composition; tris (121 g.), $MgCl_2 \cdot 6H_2O$ (203.3 g.), 800 ml. of a solution of 14.61 g. of EDTA in 4 l. adjusted to pH 8.0 with NaOH; distilled water to 100 l. The suspension was centrifuged again and the resulting slurry was frozen and stored at -20°.

The DNA-dependent RNA polymerase (EC 2.7.7.6) was prepared by the method of Chamberlin & Berg (1962). The procedure was followed through the streptomycin and protamine precipitations, and the $(NH_4)_2SO_4$ fractionations. The chromatography on DEAE-cellulose was not found to be necessary and the enzyme pellet from the final $(NH_4)_2SO_4$ precipitation was dissolved in 2.8 ml. of storage medium [$(NH_4)_2SO_4$ (0.1 M); tris-HCl, pH 8.1 (50 mM); EDTA (0.1 mM); glutathione (5 mM); $MgCl_2$ (1 mM)] and stored in 2 ml. glass vials in 0.2 ml. portions at -70°.

Enzyme dilutions were made with a solution containing: tris-HCl, pH 7.9 (10 mM); $MgCl_2$ (10 mM); β -mercaptoethanol (10 mM); EDTA (0.1 mM); bovine serum albumin (1 mg./ml.). Bovine serum albumin (fraction V; Armour Pharmaceutical Co., Eastbourne, Sussex) was also used as a standard for protein estimations (Lowry, Rosebrough, Farr & Randall, 1951) during the isolation of the enzyme.

ATP, CTP and GTP were obtained from L. Light and Co. Ltd., Colnbrook, Bucks., UTP (grade Sigma) and yeast RNA (commercial grade) were from the Sigma Chemical Co., St Louis, Mo., U.S.A., and $[8-^{14}C]$ ATP disodium salt was from Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A. Membrane filters of 0.5–1.0 μ porosity were obtained from Oxoid Ltd., London, E.C. 4.

Assay of RNA polymerase activity. Polymerase activity was determined by measuring the amount of radioactive precursor rendered acid-insoluble as described by Nicholson & Peacocke (1966). The incubation mixture contained in 0.25 ml.: tris-HCl buffer, pH 8.0 (10 μ moles); $MgCl_2$ (1 μ mole); $MnCl_2$ (0.25 μ mole); CTP, UTP and GTP (each

0.1 μ mole); $[8-^{14}C]$ ATP (0.1 μ mole and 50000 counts/min.); β -mercaptoethanol (3.0 μ moles). Enzyme and either DNA or DNH were added as described under the individual experiments. One enzyme unit is taken to be the amount of enzyme catalysing the incorporation of 1 μ mole of AMP/10 min. in the presence of 30 μ g. of DNA under the conditions of assay. Enzyme mixtures were normally incubated for 10 min. at 37° and the process was terminated by heating at 100°, followed by chilling to 0° and the addition of 0.2 mg. of carrier RNA and trichloroacetic acid to a final concentration of 5% (w/v). The precipitated material was isolated by filtration and then counted. Incorporation of radioactivity was found to proceed linearly with time and with enzyme concentration. Other features of the system have been described by Nicholson & Peacocke (1966).

Preparation of DNH. DNH was prepared from stored frozen calf-thymus glands essentially by the method of Zubay & Doty (1959) (for details see Lloyd & Peacocke, 1966).

Preparation of DNA. DNA was prepared from calf thymus in two ways. (i) DNA was prepared by the method of Kay, Simmons & Dounce (1952), followed by removal of the protein by the chloroform-gel method. This DNA was used to prepare the standard solution for enzyme assay purposes (Nicholson & Peacocke, 1966). (ii) DNA for the irradiation experiments was prepared from the gel fraction of the DNH preparation. The gel was dispersed in M-NaCl and the protein precipitated with sodium dodecyl sulphate. The DNA was precipitated with ethanol, redissolved in mM-NaCl, treated again with sodium dodecyl sulphate and the last traces of protein were removed by the chloroform-gel method. The purified DNA was precipitated with ethanol as above, redissolved in 0.7 M-phosphate buffer and dialysed against the same solvent. The final concentration was adjusted to be the same as that of the DNA in the DNH solution.

Irradiation procedures. These were as described by Lloyd & Peacocke (1966). Solutions of DNA or DNH (approx. 0.5 g./l.) were irradiated in the presence of oxygen with γ -rays (1–1.3 Mev) from a ^{60}Co source (3000 c). Dose rates were between 121 and 188 rads/min. Solutions were stored at 2° for 24 hr. (or were dialysed at 2° for about 24 hr. against the buffers described below) before adding to the incubation mixture containing RNA polymerase.

RESULTS

Incorporation of $[8-^{14}C]$ AMP by a fixed amount of enzyme increased as the concentration of the DNA increased to a limit corresponding to complete saturation of the enzyme with DNA. The general form of this curve has been described previously (Fig. 1 in Nicholson & Peacocke, 1966). For the purpose of the experiments described below, the concentrations of DNA used in the assays, either as free DNA or as DNH, were restricted to values corresponding to the lower part of the curve so that differences in priming ability might be readily detected. In practice, none of the assays contained more than 10 μ g. of DNA/0.25 ml. of assay medium.

Table 1. Incorporation of $[8-^{14}\text{C}]\text{AMP}$ into acid-precipitable material by the action of *E. coli* RNA polymerase when primed by DNA or DNH (irradiated or non-irradiated) in the presence of various buffers

The solvent during irradiation was 0.7 mM-phosphate in all experiments. The 'solvent for primer' is the solvent against which the primer (whether irradiated or not) was dialysed before adding to the assay medium, which contains tris-HCl buffer, pH 8.0 (details in Nicholson & Peacocke, 1966). No dialysis step was needed when the solvent for primer was 0.7 mM-phosphate. The reproducibility of the assay was within $\pm 4\%$ of the mean. All values are the means of duplicate determinations.

Primer	Dose of γ -rays (ev/P atom)	Solvent for primer	Units of enzyme	Incorporation (counts/min.)	Relative activity of DNH primer and DNA primer (DNH/DNA) (%)
DNH 2	8.0	0.7 mM-Tris-HCl	7.4	680	—
DNH 2	8.0	0.7 mM-Phosphate	7.4	737	—
DNH 1	—	15 mM-NaCl-1 mM-citrate	8.35	1566	48
DNA*	—	15 mM-NaCl-1 mM-citrate	8.35	3240	
DNH 2	—	0.7 mM-Tris-HCl	7.4	1440	61
DNA*	—	0.7 mM-Tris-HCl	7.4	2370	
DNH 3	—	0.7 mM-Phosphate	2.9	322	33
DNA†	—	0.7 mM-Phosphate	2.9	977	

* Prepared by method (i) (see the text).

† Prepared by method (ii) from DNH 3 (see the text).

Fig. 1. Effect of γ -irradiation on the priming ability of DNH 2 for RNA polymerase.

The conditions of assay (other than those described by Nicholson & Peacocke, 1966) for the different preparations were as follows. DNH 1 was irradiated with doses of up to 5 ev/P atom. Then 10 ml. of each sample was dialysed against flowing 15 mM-sodium chloride-1 mM-sodium citrate for 18 hr. The final concentration of nucleohistone after dialysis was 0.48 mg./ml. and each assay mixture contained 9.6 $\mu\text{g.}$ of DNA and 8.35 enzyme units. DNH 2 was irradiated with doses up to 16.0 ev/P atom. It was then dialysed against 0.7 mM-tris-hydrochloric acid buffer, pH 6.8, for 36 hr. The final concentration of nucleohistone after dialysis was 0.366 mg./ml. and each assay contained 7.32 $\mu\text{g.}$ of DNA and 7.35 enzyme units.

Both the DNH (0.4 mg./ml.) and the DNA (0.2 mg./ml.) prepared from DNH 3 were irradiated

at doses up to 16 ev/P atom. Then 0.04 ml. of each sample, not dialysed, containing 8 $\mu\text{g.}$ of DNA, was assayed with 2.9 enzyme units.

Table 1 gives some examples of the radioactivity incorporated in the presence of irradiated and non-irradiated samples of DNA and DNH, some of which had been dialysed against tris or saline-citrate buffers. As might be expected, the presence of orthophosphate did not inhibit the polymerization, which proceeds with the production of pyrophosphate.

The priming ability of DNH was between one-third and two-thirds of that of DNA, depending on the preparation studies.

Irradiated primers. The effect of irradiation on the ability of DNH to serve as a primer is shown in Figs. 1 and 2(a), which refer to DNH 2 and DNH 3 respectively. The activity remaining after irradiation, plotted as a fraction of the activity of the unirradiated DNH, decreased on irradiation. The DNA prepared from DNH 3 was irradiated at the same concentration of moles of deoxyribonucleotide/l. as the DNH 3, and their priming abilities relative to non-irradiated samples are compared in Fig. 2(a). The DNA underwent a more rapid initial loss of activity than the DNH, followed by a gradual decrease.

DISCUSSION

Decreases in the priming ability of DNA in the RNA polymerase system as a result of irradiation by X-rays have been reported by Harrington (1964) and by Zimmermann *et al.* (1964), and the present

Fig. 2. Effect of γ -irradiation of DNH 3 and of DNA on (a) the priming ability for RNA polymerase, (b) the molecular weight and (c) the hydrogen-bonded structure. M_0 and M_R are molecular weights before irradiation and after dose R respectively. β is the fraction of total number of hydrogen bonds that have been broken. ●, DNH; ▲, DNA. Data in (b) and (c) are from Peacocke & Preston (1960*a,b*) and Lloyd & Peacocke (1963*b*) for DNA, and from Lloyd & Peacocke (1966) for DNH.

results on DNA agree quantitatively with their observations. Although the ability of DNH to prime the synthesis of RNA by RNA polymerase is less than that of the corresponding amount of DNA, it is evident from Fig. 2(a) that the DNH lost this ability as a result of γ -irradiation much less readily than did DNA. Indeed, the priming abilities of DNA and DNH were the same after doses of γ -rays to each of about 1 ev/P atom. This smaller sensitivity of DNH to irradiation, compared with DNA, is paralleled by the smaller relative decrease in molecular weight and loss of secondary structure that occurs in DNH on irradiation. The conditions under which the irradiation of DNH was effected (0.7mm-phosphate as solvent, presence of air, dose rates etc.) had been chosen to be as close as possible to those prevailing in previous experiments (Peacocke & Preston, 1960*a,b*; Lloyd & Peacocke, 1963*b*, 1966) on the effects of γ -irradiation on the molecular weight and helical structure of

Fig. 3. Relationship between the activity of DNH and of DNA for RNA polymerase and the molecular weight for various γ -irradiated samples. ●, DNH; ▲, DNA.

DNH and of its DNA. In these experiments, the changes in molecular weight and in the fraction of hydrogen-bonded base pairs (β) were shown to depend only on the dosage, R , when this is expressed as ev/P atom of the DNH, and the changes observed were reproducible. It was therefore worth while to compare the changes with irradiation in the priming ability of the DNH with the changes in its molecular weight and in β that could be calculated from the known relation between these quantities and dosage, R (Figs. 2*b* and 2*c*). The relation between priming ability and molecular weight can then be inferred (Fig. 3).

Since DNA is the priming agent for RNA polymerase in the DNH, this implies that the same decrease in the overall molecular weight of DNA and DNH does not correspond to the same degradation of the DNA within the DNH. More precisely, not all the decrease in molecular weight of the DNH is to be attributed to degradation of the DNA. Some process other than degradation of DNA must also be contributing to the fall in average molecular weight of DNH that γ -irradiation produces, and this process must be of such a kind as not to impair (and even to enhance) the priming ability of the DNA in the DNH. The combined observations depicted in Fig. 3 are therefore consistent with the proposal that during irradiation the histone is dissociated from the DNA core of the DNH complex (Peacocke & Preston, 1961; Lloyd & Peacocke, 1966). During irradiation the DNA core of the DNH is both degraded and denatured (Lloyd & Peacocke, 1966), but the loss of polymerase-priming ability so induced in these experiments must be partially compensated by removal of the inhibitory histone, which is responsible for the diminished

priming ability of intact DNH as compared with DNA.

A similar comparison of the priming ability of DNA and DNH as a function of the fraction of hydrogen bonds remaining intact after irradiation is not at present possible, because of the restricted data available for the rate of rupture of hydrogen bonds in DNH. The hydrogen bonds in double-helical DNA are very sensitive to γ -rays since one internucleotide break severs the hydrogen bonds joining 10–20 base pairs on each side of the break. However, the hydrogen bonds in the DNA when it is surrounded by histone in DNH are less sensitive to γ -irradiation in solution, judging by the dose required to break a given proportion (β) of hydrogen bonds (Fig. 2c). Hence in a plot of priming ability against the number of hydrogen bonds left intact (i.e. $1 - \beta$), the DNH appears to be more sensitive than does DNA. However, this is really a reflection of the relative insensitivity to irradiation of the hydrogen bonds in DNH, and not of a closer dependence of the priming ability of DNH on its hydrogen-bonded structure. No definite conclusion about this factor can be drawn in the absence of more detailed quantitative results on the effect of γ -irradiation on the hydrogen bonds in DNH.

As mentioned above, the present results contrast with those of Weiss & Wheeler (1964), who observed, after an initial decrease with a dose of 2.1ev/P atom, an increase in priming ability of irradiated DNH, at doses from 2.1 to 12.6ev/P atom, to a level above that of the original DNH. They interpreted this in terms of the dissociation of histone that the physicochemical evidence had already suggested. In the present study, a similar inference is made from a comparison between the decrease in molecular weight and the decline in priming ability of DNH with dose. Two processes apparently occur as a consequence of γ -irradiation of DNH that affect its priming ability for RNA polymerase: (i) loss of histone, which uncovers DNA and would, if acting alone, increase the priming ability; (ii) degradation of the DNA within the DNH, which causes a loss in priming ability.

In the present experiments with the *E. coli* RNA polymerase, (ii) outweighs (i) at all stages, but it seems that Weiss & Wheeler (1964) were able, with the *M. lysodeikticus* RNA polymerase, to detect (i) before (ii) predominated. (Presumably a point would have been reached in their studies when the DNA, free of histone, would have begun to lose priming ability again.) The difference between the two sets of observations appears to be attributable to a difference in the relative rates, with respect to dose, of (i) and (ii).

Any effect of ionizing radiation that alters the control of the transcription process of DNA-to-RNA-to-protein, as would removal of histone from

the DNA, is clearly of considerable radiobiological interest. At present the problem seems to be to dissociate this effect from the more generally degradative and disruptive effects of the radiation on the DNA within the DNH complex.

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