STUDY OF X-IRRADIATED DNA USING FORMAMIDE GRADIENTS

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SUMMARY -

A density gradient of 70-100 % (v/v) formamide was prepared for the study of both native and denatured bacterial DNA. Random single- and double-stranded breaks were introduced by treating the DNA with various doses of X-irradiation. A comparison was then made between two techniques of denaturation : by heating to 100° C followed by immediate cooling, and by gentle heating in high concentrations of formamide. No significant difference was found between the molecular weights of DNA molecules thus denatured. However, the development of this gradient provides a useful tool for centrifugation studies of denatured forms of DNA in a neutral, rather than alkaline environment.

INTRODUCTION -

It has been shown (Bopp and Hagen, 1970) that during the course of denaturation of irradiated DNA using the classical technique of alkaline treatment, new single-strand breaks occur at sites where bases have been altered. It was estimated that the number of resulting breaks after denaturation was a factor of 1.5 times greater than the number of breaks caused directly by irradiation. New single-strand breaks are also introduced in the case of irradiated DNA which has been denatured by heating to 100° C followed by immediate cooling to 0° C (Rebeyrotte, 1964; Thorsett and Hutchinson, 1971). Thus, in determining the molecular weight of irradiated DNA by centrifugation in alkaline sucrose gradients, an accurate estimate of radiation damage cannot be obtained, due to the fact that new breaks are introduced by the denaturation technique itself. Knowing that in a formamide solution, native DNA will be completely denatured without the introduction of single-strand breaks (Ts'O et al, 1962), we began an investigation to determine whether or not new strand breaks would be introduced when dealing with X-irradiated DNA.

MATERIALS AND METHODS -

Escherichia coli B3 (th^-) was grown to a final concentration of 5 x 10^8 cells/ml in E medium supplemented with 8 % glucose and

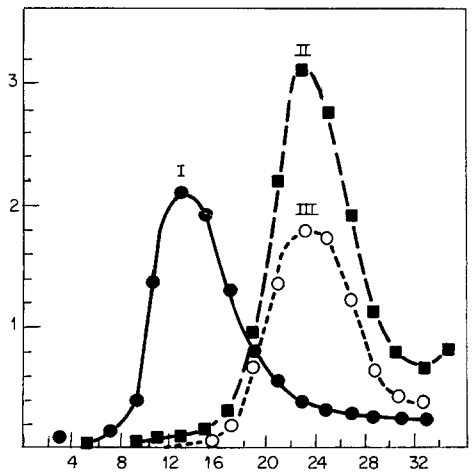

Figure 1 -

0.05 ml samples of the DNA solutions were layered on 70-100 % formamide gradients and centrifuged for 3 hours at 45000 rev/min, 18°C.
Curve I : untreated control DNA
Curve II : non-irradiated, formamide-denatured DNA
Curve III : non-irradiated, heat-denatured DNA

2 g/ml thymidine. ^{3}H-DNA was prepared by adding ^{3}H-6-thymidine (spec. act. 8 Ci/mM) present at a concentration of 2 C/ml. Cells were washed two times in SSC (0.15 M NaCl, 0.015 M Na Citrate, pH 7.0) and resuspended in 27 % sucrose in SSC. DNA was extracted according to the method of Thomas, Berns, and Kelly (1966). The final DNA concentration was approximately 40 g/ml in SSC.

The X-ray source was a Holweck tube emitting at an average wavelength of 0.9 Å, delivered at 14,000 R/min.

Denaturation was performed by one of two methods: 1) heating in SSC to 100° C for 5 minutes followed by immediate cooling in a 0° C ice bath; 2) heating in 90 % formamide to 37° C for 20 minutes, then diluting with SSC to a final formamide concentration of 65 % for layering on gradients.

Centrifugations were carried out on gradients of 70-100 % (v/v) formamide at 45,000 rev/min and 18° C for various times (SW56 rotor, Spinco L2 65B centrifuge). The same results were obtained whether or not the formamide was mixed with activated carbon before forming the gradient. After centrifugation, the bottom of each tube was pierced and 5-drop samples were collected directly onto strips of No. 17 Whatman Chromatography Pper, dried, and counted in a toluene-based scintillation liquid (4.0 g/l PPO, 0.1 g/l POPOP).

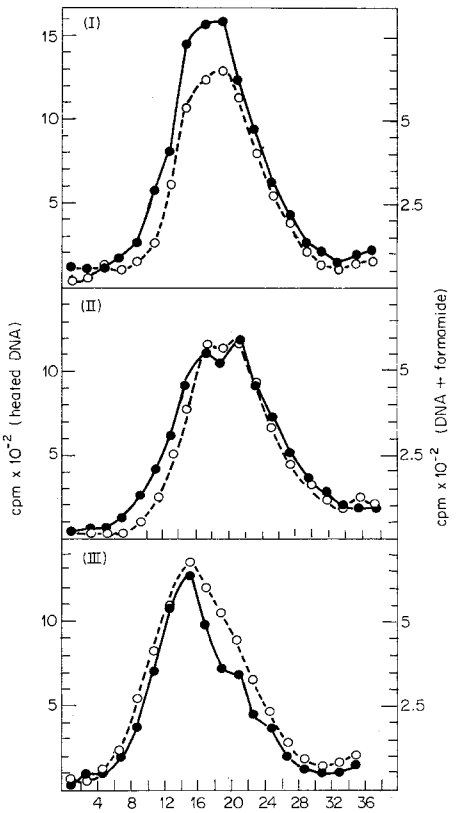

Figure 2 -
0.1 ml samples of the DNA solutions were irradiated at room temperature and subsequently denatured. The solid line represents denaturation by formamide and the dotted line represents heat denaturation. 0.05 ml samples were layered on 70-100 % formamide gradients and centrifuged at 45000 rev/min, 18° C.
Curve I : non-irradiated control (3 hr centrifugation)
Curve II : 10 kR irradiation (12 hr centrifugation)
Curve III : 30 kR irradiation (15 hr centrifugation)

RESULTS -

Non-irradiated control samples of native, formamide-denatured, and heat-denatured DNA were centrifuged in formamide gradients. Curve I of Figure 1 shows that DNA, not denatured prior to being layered on the gradient, is not denatured during the course of centrifugation. A comparison of curves II and III shows that the difference in denaturation technique had no marked effect on the resulting average molecular weight of the non-irradiated denatured DNA molecules. Formamide denaturation, however, aften yielded a sharper peak than that obtained by heat denaturation. Similar results were found using DNA from bacteriophage T7.

In a second experiment, samples of native DNA were exposed to various doses of X-radiation. Immediately after irradiation, half of the sample was denatured by formamide, and half was denatured by heating. Samples were then centrifuged in formamide gradients. The results given in Figure 2 show that the difference in the method of denaturation had no significant effect on the irradiated and subsequently denatured samples. Samples exposed to γ-radiation from a ^{60}Co source yielded similar results.

DISCUSSION -

Classical methods of DNA denaturation, such as alkaline treatment and heating, introduce strand breaks into the native DNA molecule. In studying X-irradiated DNA, we sought a milder method of denaturation so that no new single- or double-strand breaks would be introduced by the denaturation method itself. Formamide gradients have been used to denature certain DNA molecules (other than native DNA) without introducing new breaks. Gaudin and Yielding (1972) used formamide gradients to distinguish between alkali-labile regions and single strand breaks in alkalated DNA. We therefore used formamide denaturation in our studies of X-irradiated DNA. Because heat-denaturation and formamide denaturation of X-irradiated DNA yielded similar results, we conclude that approximately the same amount of chain breaks are caused by formamide treatment as by heating.

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