ON THE ACTIVITY OF INTRACELLULAR DNASE OF ESCHERICHIA COLI B AFTER X-IRRADIATION

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A recent paper by Stuy (1960) as well as the work from our laboratory (Miletić et al., 1961) have demonstrated that in microorganisms a degradation of DNA takes place during some 15-20 minutes after X-irradiation. On the other hand, a number of investigators (Kowlessar et al., 1954; Goutier-Pirotte, 1956; Kurnick et al., 1957, 1959; and others) have observed an activation of DNase in animal tissues a few hours or days after total-body X-irradiation.

Miletić et al. (1961) have found that the irradiation of a log-phase culture of E. coli B by 16.000 r X-rays results in a degradation of 15-20 % of the intracellular DNA. Our intention was to verify whether the observed degradation of DNA immediately after X-irradiation was a consequence of the activation of DNase.

A log-phase culture of E. coli B, grown in Fraser's glycerol "D" medium (Fraser, Jerrel, 1953) was harvested by centrifugation at an optical density of about 0.200. The cells were transformed into spheroplasts, as essentially described in an earlier work (Drakulić, 1959), with some modifications. The suspension of spheroplasts was divided into two equal parts which were centrifuged. One of them was retained as a control, and the other irradiated by 16.000 r X-rays.

The lysates for the measurement of DNase activity were obtained by resuspending the irradiated and the

untreated spheroplasts in ice-cold bi-distilled water immediately after irradiation. The lysates were freed from whole cells and spheroplasts by centrifuging in the cold at 3500 *g* for 30 minutes.

For the measurement of DNase activity two volumes of the lysates were incubated with one volume of 0.1 M Tris buffer (pH 8.5) containing 0.05 M $MgSO_4$, and with one volume of a solution of highly polymerized DNA (1 mg/ml) for two hours at 37°C on a rotatory shaker. Two blanks were run simultaneously under the same conditions, in one of which the lysate, and in the other the substrate, were replaced by a corresponding volume of distilled water. The reaction was stopped by chilling the incubation mixture quickly and by adding concentrated perchloric acid to a final concentration of 0.25 N. After standing in the cold for 30 minutes, the precipitate was spun down. Acid soluble deoxyribose was determined in the supernatant by the diphenylamine method, as modified by Burton (1955).

Enzyme activity was expressed in micrograms of acid soluble deoxyribose per mg of proteins. The determination of the proteins was performed in aliquots of the original lysate by the method of Lowry et al. (1951). The values for acid soluble deoxyribose obtained in both blanks were substracted from the values obtained with the whole incubation mixture.

From the results presented in Table I it is evident that no significant differences exist between the DNase activity of the irradiated and the unirradiated samples.

Work in progress in our laboratory seems to indicate also that no activation of DNase takes place in the lysates of X-irradiated cells which were incubated with or without chloramphenicol for 15 to 40 minutes after irradiation.

The above results concern the activity of alkaline

TABLE I

Experiment No.	$A_{control}$	$A_{irradiated}$	A_{irr} / A_{co}
1.	34.20	42.20	1.23
2.	34.20	49.05	1.43
3.	34.20	43.50	1.27
4.	54.00	80.85	1.50
5.	54.00	56.85	1.05
6.	54.00	51.30	0.95
7.	52.80	51.15	0.97
8.	70.80	64.05	0.90
9.	68.25	64.80	0.95
10.	61.05	63.60	1.04
11.	60.45	51.30	0.85
12.	67.05	52.80	0.79
$\bar{x}$			1.08 ± 0.23
t			0.12
p			> 0.05

$A_{control}$ = micrograms of acid soluble deoxyribose per mg of protein in untreated samples

$A_{irradiated}$ = micrograms of acid soluble deoxyribose per mg of protein in irradiated samples

$\bar{x}$ = mean value

t = test of Student

p = significance

DNase. We could not detect the activity of DNase in the acid pH range in our lysates, although we tried to liberate enzyme activity by pre-incubation with RNase (Kozloff,

1953), urea (Elson, 1959) and by prolonged incubation at 37°C.

In view of our results it does not seem likely that the degradation of DNA in E. coli B, as found by Stuy and in our laboratory, would be the result of the activation of DNase. It could be assumed instead that a damage to the DNA-replication mechanism is responsible for the observed effect.

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