X-RAY INDUCED BINDING OF N-ETHYLMALEIMIDE TO DNA IN AQUEOUS SOLUTION

Ivar Johansen, John F Ward,[1] Knut Siegel and Arild Sletten

Norwegian Defence Research Establishment, N-2007 Kjeller, Norway

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N-ethylmaleimide (NEM), when present during irradiation, sensitizes anoxic cells to the lethal effects of ionizing radiation (Bridges 1960). The effect of NEM at the cellular level may in part be due to fast radiation chemical reactions, since sensitization of anoxic bacteria is evident when NEM is added only 4 milliseconds before the irradiation (Adams et al. 1968). Oxygen may affect cellular radiosensitivity in a similar way since aerobic cells are not further sensitized by NEM (Bridges 1961). Oxygen is known to increase radiation induced damage to cellular DNA (Boyce and Tepper 1968). The present investigation was initiated to test the possibility that NEM can react with DNA during X-irradiation. A model system, DNA in aqueous solution, was used.

A dose dependent binding of ^{14}C activity to DNA was found when deoxygenated solutions of DNA were X-irradiated in the presence of ^{14}C labelled NEM. No binding occurred in the presence of oxygen. Analysis of the kinetics of complex formation suggested that the reaction occurred via a DNA radical and an NEM molecule.

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[1] Present address: Laboratory of Nuclear Medicine and Radiation Biology, 900 Veteran Avenue, University of California, Los Angeles, California 90024, USA.

Methods

Escherichia coli K-12 was labelled in DNA by growth in medium EM9 supplemented by deoxyadenosine and (methyl-^{3}H) thymidine of high specific activity (Boyce and Setlow 1962). The cells were lysed, and DNA isolated according to the method of Marmur (1961). About 1 mg of DNA (Burton 1956) at specific activity 1.7 - 2.2 x 10^{4} dpm per μg of DNA was obtained per liter of stationary phase cell culture. The amount of protein present varied between 1 and 4 % as determined according to Lowry et al. (1951). Less than 1 % RNA was present in the DNA preparation as determined from the measured amount of UV absorbing material made acid soluble by alkaline hydrolysis. To reduce binding of N-(1 - ^{14}C) ethylmaleimide (^{14}C-NEM) to DNA before irradiation, non-radioactive NEM at 400 μM was added to the DNA solution. The DNA solution was dialyzed over night vs 2 changes of 100 volumes of 0.13 M NaCl solution to wash out free NEM and ethanol present from the isolation procedure.

^{3}H-TdR labelled DNA (^{3}H-DNA) from 25 μg/ml to 400 μg/ml in aqueous solution of 0.13 M NaCl was X-irradiated in a pyrex glass vessel. The dose rate in 10 ml of solution was determined from the oxidation of ferrous sulphate and was found to be 2.7 krads/min. The DNA solution was pretreated with nitrogen or oxygen for 20 minutes by passing the gas through the vessel while the solution was stirred. The gas treatment was continued during irradiation. ^{14}C-NEM in water of specific activity 1.31 mCi/mmole (obtained from New England Nuclear) was added 5 minutes prior to the onset of radiation. Samples were withdrawn after various X-ray doses. To wash away free NEM from the mixture, DNA was precipitated by addition of ethanol, centrifuged, and the pellet dissolved in distilled water. An equal volume of 10 % cold trichloroacetic acid was added, and the precipitated DNA centrifuged down. The pellet was rinsed with ether/ethanol, and dissolved in 0.25 ml hyamin. Ten ml of liquid scintillator was added and the ^{3}H and ^{14}C activities counted.

Results and Discussion

^{3}H-DNA in aqueous solution of 0.13 M NaCl was X-irradiated in the presence of ^{14}C-NEM. Free NEM was washed away after irradiation, and the ^{14}C activity associated with the ^{3}H activity was measured. Results from a typical experiment are shown in Figure 1. A dose dependent binding of ^{14}C activity to the DNA is evident in deoxygenated solutions. No binding of ^{14}C-NEM to the DNA occurs in the presence of oxygen.

The dependence of the amount of ^{14}C-NEM bound to ^{3}H-DNA on the concentrations of DNA and NEM present during irradiation in deoxygenated solutions was determined in a series of experiments. The results are shown in Figure 2, where the reciprocal of the G value (number of molecules NEM bound to DNA per 100 eV absorbed energy) is plotted as a

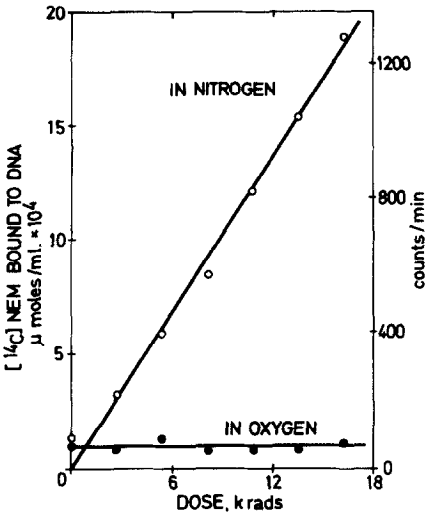

Figure 1. X-ray induced binding of NEM to DNA. ^{3}H-DNA at 200 μg/ml in aqueous solution of 0.13 M NaCl was X-irradiated in nitrogen and in oxygen in the presence of ^{14}C-NEM at 100μM. Unbound NEM was removed, and the ^{14}C activity associated with the ^{3}H activity measured in a liquid scintillation counter. The ^{14}C activity was counted with 45% efficiency and with no contribution from the ^{3}H activity present. More than 5 x 10^{3} counts were collected for each sample. The ^{3}H activity was counted with 25% efficiency and each sample gave more than 10^{4} counts/min. Less than 0.3% of these counts originated from the ^{14}C activity present, and no correction for this was made.

function of the ratio of the concentration of NEM to the concentration of DNA. The experimental points fit a straight line with an intercept of + 1 on the ordinate.

Since the binding of NEM to DNA in deaerated aqueous solution was induced by irradiation the reaction probably involve radical species. Under these experimental conditions DNA radicals and NEM radicals are formed mainly by reactions with radiation induced water free radicals. The number of the DNA radicals versus the number of the NEM radicals formed depends upon the relative concentration of the two solutes and their reactivity toward water free radicals. Thus an increase in DNA

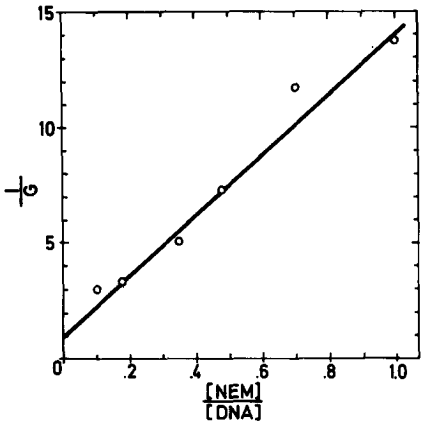

Figure 2. X-ray induced binding of ^{14}C-NEM to ^{3}H-DNA in deoxygenated solutions as a function of the molar concentration of NEM to the molar concentration (with respect to phosphate) of DNA. The loss of NEM from solution due to evaporation was negligible during these experiments.

concentration relative to NEM concentration will result in a higher proportion of DNA radicals formed. This will result in a higher yield of complex formation if the binding is between a DNA radical and an NEM molecule, but a lower yield of complex formation if the binding is between a DNA molecule and an NEM radical. It was found that G(NEM bound) increased with increasing (DNA)/(NEM). If the binding is by a DNA

radical and an NEM radical the complex formation will reach high value at a certain ratio of (NEM)/(DNA), in which any change will lead to a lower yield of complex formation. In the present experiments the ratio (NEM)/(DNA) was varied from 0.1 to 1.0 and the results obtained do not support this mechanism. If simple kinetics is assumed the expression

$$\frac{1}{\alpha} = 1 + \frac{1}{k} \frac{(NEM)}{(DNA)}$$

is obtained where k is a constant, and α is the fraction of water free radicals reacting with DNA. The experimental points in Figure 2 fit a straight line when 1/G (NEM bound) was plotted against (NEM)/(DNA). This suggests that the G value of complex formation is directly related to α, and that the complex formation is by a DNA radical reacting with an NEM molecule. This would be in agreement with the finding that oxygen prevents this reaction (Figure 1), since it is widely assumed that oxygen reacts readily with organic free radicals.

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