Radiation-Induced Binding of 4-Nitroquinoline-N-Oxide to DNA in Aqueous Solution

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4NQO-DNA mixture solution was irradiated with 60Co gamma-rays. Binding yield of 4NQO to DNA was compared to that of cysteine to DNA after fractionation by gel filtration. Their binding yields increased with increase in radiation dose. The dose vs binding yield curves of cysteine to single stranded DNA, cysteine to double stranded DNA, and 4NQO to double stranded DNA showed first order kinetics, but that of 4NQO to single stranded DNA was found to be different from the others i.e. it showed that the reaction was a second power function of radiation dose.

INTRODUCTION

4NQO (4-nitroquinoline-N-oxide), a chemical carcinogen, is structurally aromatic and has a characteristic NO group. The presence of noncovalent crosslinking in vitro between 4NQO and DNA without radiation has been reported.¹,² A study has been made by Tada et al. on the binding of 4NQO to DNA promoted with UV (personal communication). However, no data is available on 4NQO-DNA binding caused by ionizing radiation. In this paper, the radiation-induced binding activity of 4NQO to DNA is compared to that of cysteine which is a high radiation-induced binding participant.³⁻⁷

MATERIALS AND METHODS

Preparation of solutions and irradiation

Calf thymus DNA (Type I) was purchased from Sigma Chemical Company, St. Louis, Mo. 4NQO-5, 6, 7, 8, 9, 10⁻¹⁴C (6.22 mCi/mmole) and L-cysteine-³⁵S (56 mCi/mmole) were obtained from Daiichi Pure Chemical Company, Japan, and Radiochemical Centre, England, respectively. 4NQO, L-cysteine, and other chemicals were of special grade and from Wako Chemical Company, Japan. Solutions were prepared with triply distilled water.
Double stranded DNA or single stranded DNA (prepared by rapid cooling method) and 4NQO or cysteine were dissolved in saline-citrate solution (0.15 M NaCl + 0.015 M sodium citrate). Final concentration was 1 mg/ml for DNA and $5 \times 10^{-4}$ M, 0.5 $\mu$Ci/ml for 4NQO and cysteine. After bubbling with N$_2$ for 15 min, a 1 ml aliquot of solutions was irradiated in Pyrex glass tube (1.2 x 9.0 cm) with $^{60}$Co gamma-rays (Shimadzu RT-10000S) at a dose rate of 10,000 rad/min at 0°C. Dosimetry was performed with the Fricke dosimeter.

Column chromatography and measurement of radioactivity

Ultrogel AcA 22 (LKB, Sweden) was loaded up to 40 cm in the Laboratory Column, K 9/60 (0.9 x 60 cm) (Pharmacia Fine Chemicals, Sweden). An aliquot of the irradiated solutions was eluted with saline-citrate solution through the column, and another aliquot was mixed with sodium dodecyl sulfate (SDS) (10% in final) and then eluted. After elution, optical density of each fraction (2.3 ml) was measured at 260 nm, and then one volume of cold 20% trichloroacetic acid (TCA) was added to the solution. The precipitate was filtered with a glass filter (Whatman CF/C), washed with 5% TCA and then dried. The radioactivity in the precipitate was measured with a Packard Tri-Carb Scintillation Spectrometer.

RESULTS AND DISCUSSION

Solution of 4NQO mixed with double stranded DNA or single stranded DNA were irradiated, and passed through an Ultrogel column, and the sample were then treated with TCA. Chromatograms obtained are shown in Fig. 1.

In a range from the third fraction to the 9th fraction which corresponds to the DNA peak area, the total $^{14}$C-radioactivity of 4NQO increased with an increasing radiation dose, which indicates the radiation-induced binding of 4NQO to DNA. $^{14}$C-radioactivity in the 4NQO peak area also increased with the increase in radiation dose. This increase may be due to the formation of a 4NQO polymer which could be more TCA insoluble than 4NQO. No report of 4NQO polymerization induced by radiation has been published.

The binding of cysteine to DNA as a function of the radiation dose is shown in Fig. 2 and can be compared with the binding of 4NQO to DNA shown in large Fig. 3. Prior to radiation, 4NQO and cysteine bound somewhat not only to double stranded DNA but also to single stranded DNA. These binding yields were not affected by incubation time at 0°C within 12 hr. When SDS was added to the solution before elution, negligible amount of 4NQO and cysteine bound to DNA (see 0 rad on dotted lines in Figs. 2 and 3). Therefore, in the absence of SDS, there is some formation of noncovalent bonds (physical binding) of 4NQO or cysteine to DNA. Reports have been published in 1966 on noncovalent binding of 4NQO to DNA without radiation. Interaction of deoxyribo-nucleosides with 4NQO was also reported.

When a mixture of 4NQO, DNA and SDS was heated in boiling water for 5 min,
the elution pattern was very complex. This shows that heating also induces a binding of 4NQO to DNA. For the cysteine-DNA-SDS mixture, its binding yield was independent of heating. Therefore, the dotted lines in Figs. 2 and 3 show their covalent binding yields at least for the cysteine-DNA mixture and probably for 4NQO-DNA solution.

The radiation dose vs binding yield curve for single stranded DNA is similar to that for double stranded DNA in the case of cysteine, but was very different in the case of 4NQO. In the 4NQO-double stranded DNA binding reaction, a linear response similar to that obtained with cysteine was observed (large Fig. 3). With cysteine, the ordinate for Fig. 2 was \( \frac{[\text{Cys}]}{[\text{Cys}]-[\text{Cys-DNA}]} \) in log scale for both single stranded DNA and double stranded DNA. With 4NQO, the ordinate in large Fig. 3 was \( \frac{[4\text{NQO}]}{[4\text{NQO}]-[4\text{NQO-DNA}]} \) in log scale for double stranded DNA. The linear plot in this case indicates first-order kinetics. However, with 4NQO and single stranded DNA, this plot is curvilinear (large Fig. 3). When the ordinate is \( \frac{[4\text{NQO}]}{[4\text{NQO}]-\sqrt{[4\text{NQO-DNA}]} \)}
in log scale, the plot becomes linear (Fig. 3 inset). The indicates that the reaction is second order in relation to radiation dose. A radiation-induced binding mechanism of the specific amino acids, aromatic amino acids and sulfur-containing amino acids including cysteine, to DNA was discussed previously. It seems that the binding mechanism of 4NQO to double stranded DNA is similar to that of the specific amino acids because of the same kinetics. The different binding reaction of 4NQO to single
stranded DNA should depend on the absence of hydrogen bonds between the two strands of DNA. It is unknown, however, whether the different reaction is due to an altered electron density distribution in the bases of the single stranded DNA compared to that in double stranded DNA, or due to stereochemical factors. Taking into consideration of a special binding form of 4NQO with the nucleic acid bases, the study of this binding mechanism is now ongoing.

Fig. 3. Dose vs binding yield curves of 4NQO to DNA. Initial concentration of 4NQO: 5 x 10^{-4} M, 0.5 μCi/ml; initial concentration of DNA: 1 mg/ml. [4NQO]: Initial concentration of 4NQO (100%); [4NQO-DNA]: binding yield of 4NQO to DNA (percent of initial concentration of 4NQO). ---: Without SDS; ----: with SDS. Ordinate is graduated in log scale.
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