

On the Influence of p-Nitroacetophenone on Radiation-Induced Molecular Size Changes of DNA

K. Washino and W. Schnabel

Hahn-Meitner-Institut für Kernforschung, Berlin GmbH,
Bereich Strahlenchemie Berlin

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DNA has been irradiated in aqueous solutions with 16 MeV electron pulses (duration: 50–100 ns). In the presence of p-nitroacetophenone (PNAP) the light scattering intensity (LSI) increased after the pulse in Ar- and O₂-saturated solutions indicating intermolecular cross-linking. The LSI increase was greatest in Ar-sat. solutions containing t-butanol (1 mol/l). In the absence of PNAP and t-butanol DNA was degraded as indicated by a decrease of LSI. The results contribute to the understanding of radiation-induced reactions, DNA undergoes in the presence of sensitizers of high electron affinity.

We would like to report on studies concerning the influence of “sensitizers” on radiation-induced changes of the molecular size of native deoxyribonucleic acid. Various sensitizers were tested, especially p-nitroacetophenone (PNAP), N-ethylmaleimide (NEM) and nifuroxime (NF). In this paper results obtained with PNAP are reported, since most experiments were carried out with compound so far. The other two sensitizers exhibit a quite similar behavior. PNAP has been reported to be capable of significantly enhancing radiation-induced damage in hypoxic mammalian cell cultures [1, 2]. According to a recent report PNAP enhances the yield of radiation produced single strand breaks in DNA as measured shortly after irradiation of *Escherichia coli* B/r in an anoxic medium [3]. Pulse radiolysis studies using optical and conductometric detection methods revealed that PNAP is highly electron affinic

$$(k_{e_{aq}} + \text{PNAP} = 4.1 \times 10^{10} \text{ l/mol s})$$

[4, 5]. In various cases efficient electron transfer from free radicals to PNAP has been detected [5].

In our experiments we have irradiated dilute aqueous solutions of calf thymus DNA with 16 MeV electron pulses (pulse duration: 50–100 ns; ab-

sorbed dose per pulse: 10 to 90 krad) under various conditions. With the aid of the light scattering detection method variations of molecular size were measured as a function of time. For further details refer to [6].

In solutions containing PNAP (5×10^{-4} mol/l), the light scattering intensity (LSI) was found to increase after the pulse, indicating an increase in the molecular size. Since a conformational change is not feasible, the LSI increase must be due to an increase of the average molecular weight, i.e. our measurements evidence intermolecular crosslinking.

In Fig. 1 the extent of LSI change is plotted vs. the absorbed dose. In accordance with earlier results [7] and with the well-known behavior of DNA in stationary irradiations with γ -rays [8] the LSI decreased in solutions containing no PNAP indicating double strand breaks. As expected, O₂ enhances double strand degradation (relative to Ar-saturated solutions) and t-butanol prevents degradation owing to the fact that OH radicals, which are responsible for strand breaks, are scavenged by the alcohol.

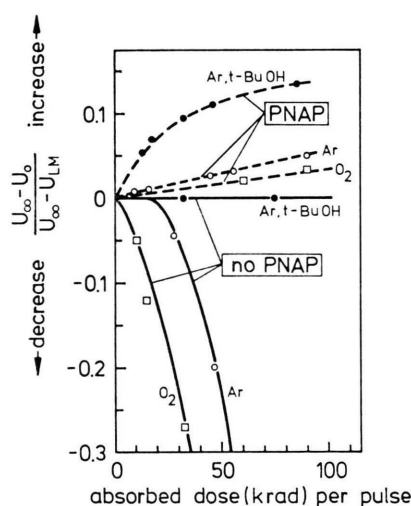


Fig. 1. The extent of light scattering intensity (LSI) change as a function of the absorbed dose per pulse of 16 MeV electrons. Pulse duration: 50–100 ns. Irradiation conditions are indicated at the curves. [t-BuOH] = 1 mol/l; in O₂ saturated solutions [O₂] = 1.3×10^{-3} mol/l. For additional conditions refer to legend of Figure 2. The ratio $U_{\infty} - U_0 / U_{\infty} - U_{LM}$ corresponds to the degree of cross-linking or degradation, respectively. U_{∞} , U_0 , and U_{LM} denote signal voltages (proportional to the LSI): a long time after the pulse (U_{∞}), before the pulse (U_0) and solvent (U_{LM}).

Reprint requests to Prof. Dr. W. Schnabel, Hahn-Meitner-Institut für Kernforschung, Berlin GmbH, Bereich Strahlenchemie, Glienicke Straße 100, D-1000 Berlin 39.

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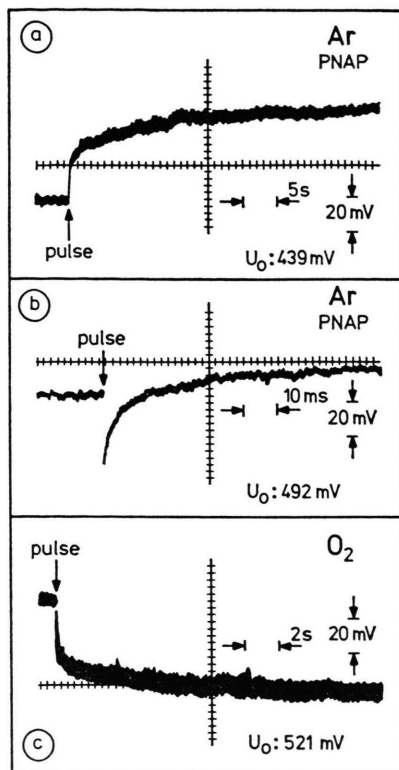
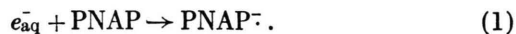


Fig. 2. Typical oscilloscope traces demonstrating changes of the LSI after the pulse observed with aqueous solutions containing calf thymus DNA (0.1 g/l, $\bar{M}_w > 10^7$) and NaCl (10^{-2} mol/l) at pH = 7.5. (a) Ar-saturated solution, [PNAP] = 5×10^{-4} mol/l, [t-BuOH] = 1 mol/l, absorbed dose per pulse: 55.7 krad. (b) Time resolved rapid mode of LSI change in trace (a). (c) O_2 -saturated solution, absorbed dose per pulse: 54 krad.

Figure 2 shows typical oscilloscope traces depicting the LSI increase in Ar-saturated solution containing PNAP and t-butanol ((a) and (b)) as well as the LSI decrease in O_2 -saturated solution containing no other additive (c). As indicated by the initial decrease of the LSI in trace (b) the optical absorption of the solution increases rapidly and decreases again within several ms. This effect was corroborated by respective optical absorption measurements. It is due to formation and decay of $PNAP^{\cdot-}$.

Whereas the elucidation of the chemical mechanism affords further work, it can be concluded from the present results that PNAP and possibly anion radicals of PNAP are principally responsible for the observed crosslinking effect. It was estimated on the basis of the rate constants of the reactions of e_{aq}^- with PNAP and DNA that at the concentrations

chosen for these experiments almost all hydrated electrons underwent the reaction



Principally, crosslinking becomes feasible only on the basis that reactive sites on DNA chains are created. From the fact that the increase in LSI in the presence of PNAP is most pronounced in Ar-saturated solutions containing t-butanol, it is inferred that indirect radiation effects do not become operative on DNA. At our reaction conditions reactive species generated in the radiolysis of water were scavenged almost completely by the additives. Apart from the fact that the reaction products of the scavenging processes might attack DNA, it appears at present most probable that the crosslinking reaction originates basically in direct action of radiation on DNA. Thus, the crosslinking effect could be interpreted in terms of the concept developed earlier [9] that PNAP and related compounds scavenge electrons generated in primary ionizations in DNA, thus preventing ion recombination and promoting other reactions. The mechanism of the crosslinking reaction has to be elucidated in more detail, especially with respect to the question whether PNAP is incorporated in DNA during the process. The interpretation of the crosslinking effect based on direct action of radiation becomes feasible because of the high molecular weight of DNA. At an absorbed dose of 5×10^4 rad, e.g., the generation of 0.5 reactive sites per macromolecule is estimated on grounds of $G(\text{reactive sites}) = 1$ and $MW = 10^7$.

The relatively weak LSI increase observed in Ar- and O_2 -saturated solutions containing PNAP might be explained in terms of a competition of crosslinking and double strand breakage. OH radical attack on sugar moieties causes strand breaks. The latter process is, however, partially inhibited because OH radicals are scavenged by PNAP.

The results obtained contribute to the elucidation of the mechanism of radiation-induced reactions of DNA in the presence of PNAP and related sensitizers.

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