Detachment of Segments from DNA Double Strands as Detected by Time Resolved Rayleigh Light Scattering

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Calf thymus DNA was irradiated in oxygen saturated 0.01 N NaCl solution with 2 μs pulses of 15 MeV electrons. By monitoring the decrease of light scattering intensity after the pulse, two modes of decrease were detected: The fast decrease ($\tau_{1/2} \approx 0.8$ ms) is ascribed to the separation of DNA fragments produced by double strand breaks at positions directly opposite to each other. The slow decrease ($\tau_{1/2} \approx 8$ s) is attributed to the detachment of segments generated by single strand breaks at sites on the alternate strands being separated by about 10 nucleotide units.

The light scattering detection method developed at the Hahn-Meitner-Institut [1] was used during recent years in combination with pulse radiolysis or laser flash photolysis to study the dynamics of synthetic macromolecules in solution. It was possible, e. g., to observe directly the disentanglement of coiled macromolecules [2, 3]. Recently evidence for preferential solvation of macromolecules could be obtained by our method [4].

In this note we wish to report on experiments carried out with calf thymus deoxyribonucleic acid (DNA). The results obtained yielded information on the rate of separation of molecular fragments produced by double strand ruptures. Furthermore, it was possible to observe directly the detachment of single strand segments from the double stranded helix. Fig. 1 depicts the well-known fact [5, 6] that double strand breaks may result either from two single strand breaks on opposite sites of the double helix or from single strand breaks separated by a number of intact nucleotide units. In the latter case double strand breaks are completed only after the detachment of the small segments in between the breaking points. One expects that in case (a) of Fig. 1 diffusion determines the rate of fragment separation. In case (b) the rate of melting of the segments between the breaking points should contribute to the rate of separation and should become
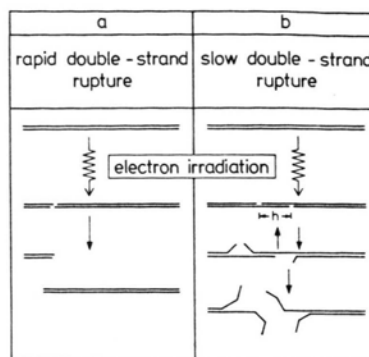
Fig. 1. Schematic description of the generation of double strand breaks in DNA.

eventually rate determining at a certain length of the segments.

The DNA ($\bar{M}_w \approx 2 \times 10^7$) used for our experiments was prepared according to Kay et al. [7]. It was irradiated at a concentration of 7.5×10^{-2} g/l in oxygen satured 0.01 N NaCl solutions with 2 μs pulses of 15 MeV electrons from an L-band linear accelerator (Vickers) at room temperature. The absorbed dose per pulse was varied between 7 and 60 krad. If the dose absorbed per pulse was smaller than about 10 krad, no change of the light scattering intensity (LSI) was observed. This is shown in Fig. 2 a. When the solution was irradiated with a second pulse, the LSI dropped rapidly (Fig. 2 b). This drop was followed by a further slow decrease of the LSI with a half life $\tau_{1/2}$(slow) ≈ 8 s. Upon irradiating the solution with a pulse of an absorbed dose greater than 10 krad the slow decrease of the LSI was observed already at the first pulse. Irradia-
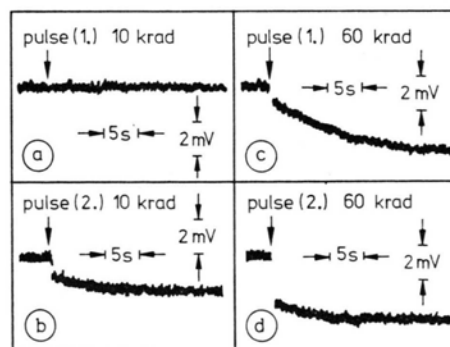

Fig. 2. Oscilloscope traces demonstrating the change of the light scattering intensity after irradiation of a DNA solution (7.5×10^{-2} g/l) at room temperature. The initial LSI corresponded to 23 mV. a. solution was irradiated with one pulse of 10 krad; b. solution was irradiated with a second pulse of 10 krad (total dose 20 krad); c. solution was irradiated with one pulse of 60 krad; d. solution was irradiated with a second pulse of 60 krad (total dose 120 krad).

tion with a pulse of more than 50 krad yielded at the first pulse already the fast decay, followed by the slow one with $\tau_{1/2}$(slow) $\approx$ 8 s, as shown in Fig. 2 c. At a second pulse the portion of the rapid LSI decay was in this case higher than at lower pulse doses. This is shown in Fig. 2 d. For the rapid LSI decay a half life $\tau_{1/2}$(fast) $\approx$ 0.8 $\pm$ 0.2 ms was evaluated from oscilloscope traces like the one shown in Fig. 3.
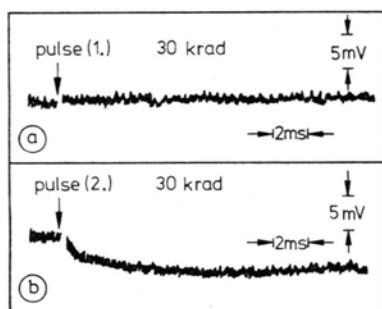

Fig. 3. Oscilloscope trace of the fast decay of the light scattering intensity observed after irradiating a DNA solution (7.5 × 10^{-2} g/l) at room temperature (pulse dose: 30 krad). The initial LSI corresponded to 53 mV. a. solution was irradiated with one pulse of 30 krad; b. solution was irradiated with a second pulse of 30 krad (total dose 60 krad).

The results are explained in the following way: a minimum absorbed dose is needed to cause a decrease of the LSI since breaks in positions as described in Fig. 1 a are formed only with a certain probability of single strand breaks in the DNA molecules. If the absorbed dose is sufficiently high, the LSI drops already after the first pulse. Otherwise several pulses are needed. The fast decrease $(\tau_{1/2}$(fast) $\approx$ 0.8 ms) is attributed to the diffusion of DNA fragments generated by double strand breaks of the type described in Fig. 1 a. In this case the fragments are assumed to separate immediately after main chain bond cleavages. The latter processes are faster than $\tau_{1/2}$(fast) $\approx$ 0.8 ms. This is concluded from the fact that $\tau_{1/2}$(fast) decreases with increasing degree of degradation. Thus $\tau_{1/2}$(fast) does not correspond to the life time of intermediates (e. g. radicals). $\tau_{1/2}$(slow) is attributed to the detachment of segments generated by single strand breaks of the type desribed in Fig. 1 b. It was found that $\tau_{1/2}$(slow) decreases significantly with increasing temperature. If $\tau_{1/2}$(slow) may be correlated to relaxation times measured by Eigen and Pörschke [8] with the temperature jump method for oligo(ribouridylic acid)-oligo(riboadenylic acid), the length of the detaching segments may be estimated to be about 10 nucleotide units. This value is in agreement with the respective values (h) obtained previously [5, 6] by sedimentation analysis. In those experiments the number h of base unit pairs which cannot prevent a double strand rupture between two opposite single strand breaks was calculated from the rates of production of single and double strand breaks and was found to be between 7 and 16.

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