ESRF	**Experiment title:** Generation of hydroxyl radicals (OH•) pulses in solution by Synchrotron radiation: A new method for fast (OH•) footprinting	**Experiment number:** LS-1413
Beamline: **ID9**	**Date of experiment:** from: 29.11.99 to: 2.12.99	**Date of report:** 21.02.00
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Report:

The aim of experiment LS-1413 was to analyze the binding kinetics of the RNA-polymerase to DNA in the sub-second time range. As the synchrotron was running in the 16-bunch mode we decided first to test the amount of radicals which were produced under the reduced flux conditions of the 16-bunch mode. Therefore solutions of free, ^{32}P labeled DNA were exposed in times between 1.5ms to 25ms to the white beam of the ID9. The DNA-solution was mixed and pushed through the beam using a quenched flow apparatus as described in previous reports & proposals. The cuvette is build of a thin quartz capillary (outer diameter: 1mm, thickness of the walls: 0.01mm). The proposed Be-window cell (see report LS-1274) was canceled, because it is not possible to obtain properly coated, water resistant beryllium windows which were necessary in order to avoid poisoning of the protein solution. The capillary was not damaged during the exposures (duration between 1.5ms to 25ms).
The result of the experiment is show in the gel electrophoresis picture in fig. 1.

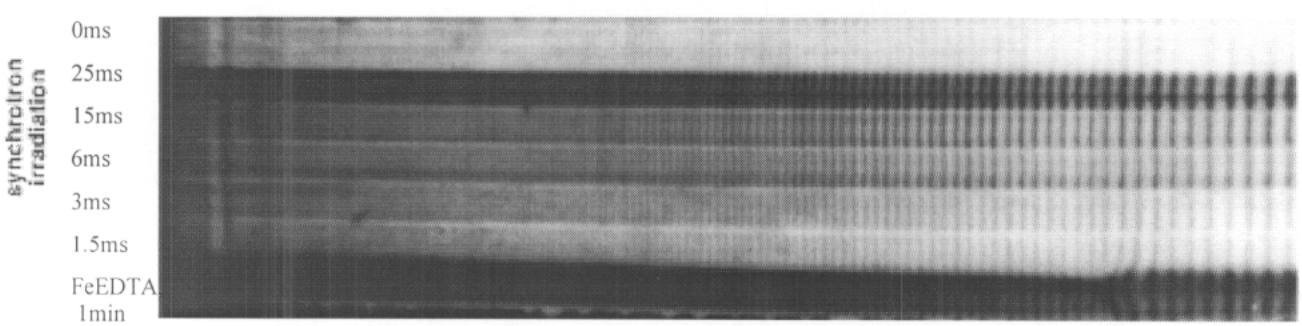

Fig. 1: Gel electrophoretical analysis of the free DNA at different exposure times. The well pronounced bands indicate good cleavage efficiency.

Further analysis was done by scanning the intensities of the cleavage bands. The result is shown in fig.2 for the 25ms exposure time.

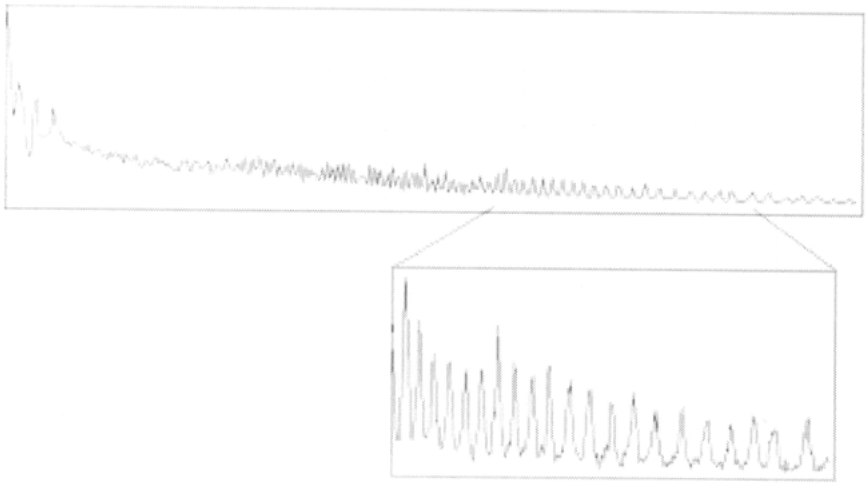

Fig. 2: Scanned band intensities of the 25ms exposure. The analysis show clear bands of cut DNA fragments. The sharp peaks having almost equal intensities (squares) indicate well OH• cleaved DNA, without any unspecific strain breaks due to direct X-ray photon hits (which are believed to be base-specific).

From this result we were able to estimate the amount of produced OH• radicals compared with chemical produced radials using the Fenton reaction with FeEDTA.

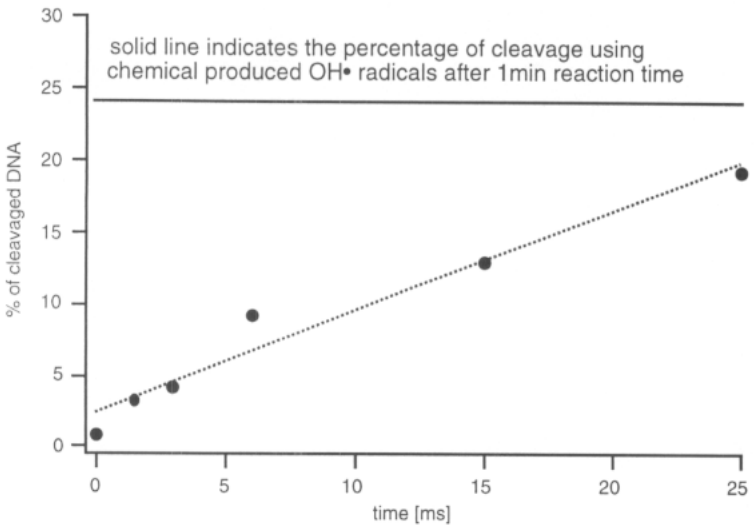

Fig. 3: Percentage of DNA cleavage by photon produced OH• radicals using white synchrotron radiation. The cutting efficiency is linear with the exposure times. The amount of cleaved DNA at 25ms exposure is 5% smaller as the highest rate obtained by chemical produced radical within 60s reaction time.

The most important conclusion is that even at very short exposure times we have rather good cleavage yields comparable with the yield of chemical cleavage allowing to study the complex rearrangement at milliseconds range, as well as the translocation of the protein during the transcription of DNA to RNA.

In fig. 4 the footprints of RNA polymerase bound to labeled DNA are shown. The protein protected DNA regions appear in the line diagram as regions with less pronounced (or even missing) bands.

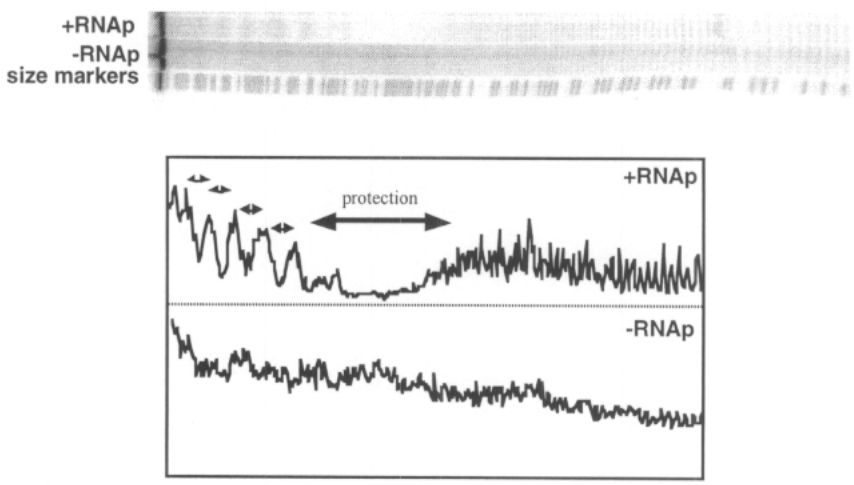

Fig. 4: Gel analysis pattern of RNA polymerase bound to ^{32}P labeled DNA using chemical produced OH•. The amount of cleavage is comparable with the amount gained by using white synchrotron radiation.