ESRF	**Experiment title:** Generation of hydroxyl radicals (OH*) pulses in solution by synchrotron radiation: a new method for fast OH-footprinting	**Experiment number:** LS869
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Report:

AIM OF THE EXPERIMENT

The aim of our approach is to use hydroxyl radicals produced by X-rays irradiation for fast kinetic studies of the rearrangements of protein-DNA complexes. We are especially interested in studying fast rearrangements of RNA polymerase - DNA complexes proceeding within time intervals 0.01-l sec. For this purpose a powerful beam is required that can generate a high flux of hydroxyl radicals sufficient to cleave DNA in such short time intervals. Unattenuated ESRF white beam of ID2 Laue beamline yielding an estimated power of 6W at the sample would fulfil1 these requirements.

EXPERIMENTAL PROCEDURE

As a first step minimal irradiation times yielding DNA cleavage had to be estimated. This was the main purpose of our experiment LS869. In order to elaborate the conditions of such cleavage, 100 μl aliquotes of the solution, containing DNA fragment (220 base pairs length, $2x10^{-8}$ M), were pumped through the quartz capillary (inner diameter 0.85 mm, outer diameter 1 mm) which was positioned in the middle of the beam. The different exposure times to the beam were achieved by applying different pumping rates (from 2.4 to 120 μl/sec). The samples were collected and analyzed by biochemical methods using facilities provided by the EMBL outstation in Grenoble.

RESULTS

The results shown in Fig.1. indicate that exposure to white beam of ID2 Laue beamline cause a DNA cleavage pattern characteristic for hydroxyl radical cleavage. The minimal detectable cleavage occures at the exposure times as short as 20-50 rnsec. Exposure to the beam for 100-200 msec give the yield of cleavage comparable with that produced by radicals generated chemically for 2 min. Despite the high power of the beam (6W) no heat

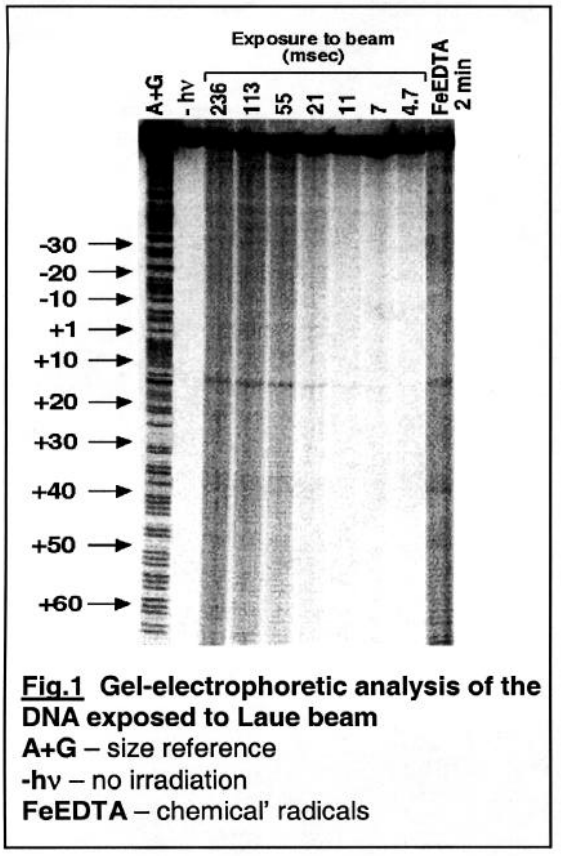

Fig.1 **Gel-electrophoretic analysis of the DNA exposed to Laue beam**
A+G – size reference
-hν – no irradiation
FeEDTA – chemical' radicals

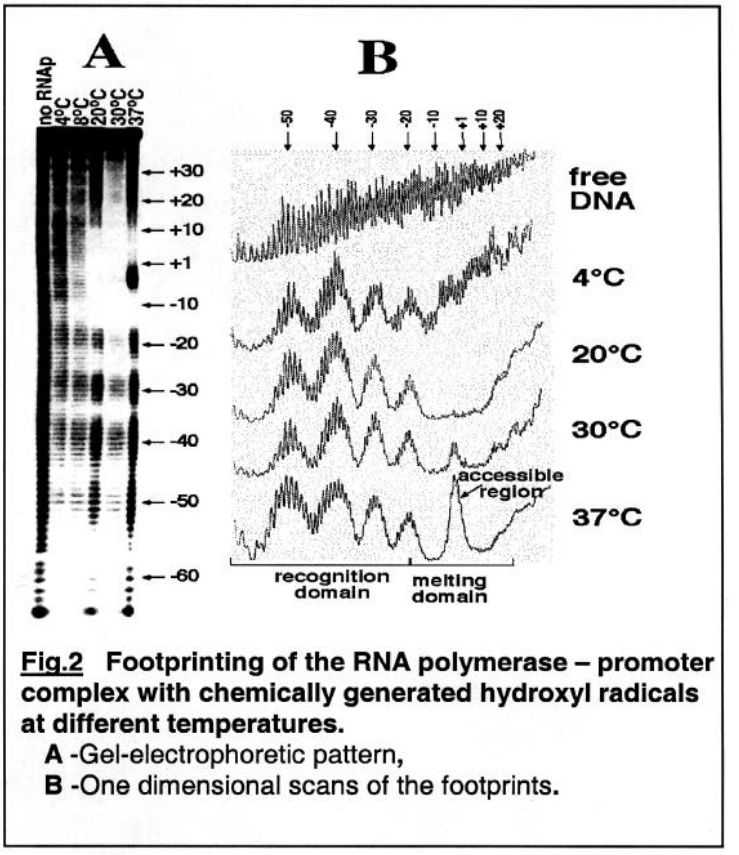

Fig.2 **Footprinting of the RNA polymerase – promoter complex with chemically generated hydroxyl radicals at different temperatures.**
A -Gel-electrophoretic pattern,
B -One dimensional scans of the footprints.

CONCLUSIONS AND PROSPECTS FOR FURTHER MEASUREMENTS.

1) OH radicals generated by white X-ray beam of ID2 Laue beamline can be used for footprinting studies on DNA.

2) The intensity of the radicals production at the beam is 2-3 orders higher than that produced by conventional chemical methods. This potentionally enables monitoring of the dynamics of the DNA-protein interactions in the timescale of about 100 msec.

3 The equipment and especially quarz capillary has been proven to be adequate.

As indicated in the proposal, the following measurements will include the footprinting studies with DNA-dependent RNA polymerase. Recent footprinting data obtained in our lab. concerns the rearrangement of RNA polymerase complex during formation of so called „open complex". As it is seen in the Fig.2, at low temperature (4°C) the enzyme binds at the DNA sequence upstream the starting point („recognition domain") protecting only one side of the DNA helix. At higher temperatures it interacts with the „melting domain" wrapping around the initially transcribed sequence (between positions -20 and +20). At the temperatures above +30° the interactions become most tight and the region around position -5 of the DNA template strand becomes accessible for hydroxyl radicals. We assume that this „temperature profile" of the open complex formation reflects the real time sequence of the events occuring in vivo. Since this process is very fast (in seconds range), the proof for our assumption can be obtained only by fast footprinting methods. Using the high intensity X-ray sourse of ESRF is an adequate solution for this problem.

We ask for support to further develop this new exciting approach which promises new