Scientific report of Viktorie Stisova for STSM 19/09-14/10/2005 in CBM CNRS Orleans

Radiation damage to DNA-protein complexes

The collaboration between the group of Radiation Biophysics in the Department of Radiation Dosimetry of the Nuclear Physics Institute (DRD NPI) and the group of Radiobiology of Nucleic Acids and Proteins in the Centre de Biophysique Moléculaire CNRS (CBM CNRS) Orleans France has a long-term tendency. The current research topics of both groups are related to the radiation damage to specific complexes between DNA and proteins. The Czech group focuses for several years on the theoretical modeling of radiation damage to biomolecules. When the predicted types and distributions of damages within DNA, proteins or DNA-protein complexes can be compared to experimental data, new information about the mechanisms underlying the biological effects of ionizing radiation can be obtained. The French group applies different techniques to study primary damages caused by ionizing radiation at molecular level experimentally. The calculated distributions of damages within DNA and/or proteins are compared with experimental data and new information about biological effects of ionizing radiation can be obtained.

A part of the experiments can be currently performed only in the group of Radiobiology of Nucleic Acids and Proteins at the CBM CNRS. Moreover, the scientific stay allows discussing problems and learning techniques of molecular biology that can be then introduced in the laboratory in Prague.

During the STSM of Viktorie Stisova in CBM CNRS a specific complex between DNA bearing the estrogen response element specific sequence and estrogen receptor protein has been studied. The two purchased 59 bp DNA complementary oligonucleotides were purified on denaturing polyacrylamide gel. Since the studied protein is very sensitive for temperature changes, and overall storage conditions, its stability was tested using native retardation gel electrophoresis. The set of experiments showing the stability of the complex under irradiation, and the formation of the complex between irradiated DNA and non-irradiated protein, or non-irradiated DNA and irradiated protein, has been reproduced to validate the experimental conditions, which were previously performed in Prague workplace.

To determine a possible footprint of the protein on the DNA strand breakage pattern, the method of sequencing polyacrylamide gel electrophoresis has been used. The experimental condition (binding buffer composition, appropriate dose range) has been tested using native retardation gel electrophoresis. Achieved results showed that due too high concentration of scavenging compounds in the binding buffer, the DNA damage is not sufficient to observe the footprint even for very high doses (2kGy).

The mentioned experiments have been performed using the biological material (DNA oligonucleotide, estradiol, tamoxifen, estrogen receptor protein, and other chemicals) purchased from the grant 1P05OC085 of the Ministry of Education, Youth and Sports of the Czech Republic, which is national financial support of the COST P9 activities, grant 10-85093 of the Czech Technical University, and grant 202/05/H031 of the Grant Agency CR. The existing equipment, material and know-how of experimental techniques in host laboratory have been used.