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REFERENCE: Short Term Scientific Mission (STSM), COST P9
Beneficiary: Joanna DIDIK, Institute of Applied Radiation Chemistry, Technical University of Lodz
Host: Peter WARDMAN, Gray Cancer Institute, Mount Vernon Hospital, Northwood, Middlesex
Period: from 11/02/2006 to 26/02/2006 Place: Gray Cancer Institute (UK)
Reference code: COST-STSM-P9-136

SCIENTIFIC REPORT

PURPOSE OF THE VISIT

It is known that radiation induces formation of the reactive oxygen and nitrogen species in the cells and tissues. The aim of my visit was to investigate the reactions of radiolytically generated carbonate radical anions ($^{\bullet}CO_3^-$) and nitrogen dioxide radicals ($^{\bullet}NO_2$) with ferric cytochrome c (Fe^{3+}). The pulse radiolysis equipment in Gray Cancer Institute allowed me to generate and observe $^{\bullet}CO_3^-$ and $^{\bullet}NO_2$ radicals at micromolar concentrations, which was not possible in my own laboratory.

DESCRIPTION OF THE WORK CARRIED OUT DURING THE VISIT

Pulse radiolysis experiments were performed with a 6 MeV linear accelerator. The doses per pulse were 2.2 or 5.8 Gy. Carbonate radicals ($CO_3^{\bullet-}$) were produced by the radiolysis of aqueous solution of 0.1 M $NaHCO_3$, saturated with CO_2 (4.2%) and N_2O (95.8%) at pH 8.0. The kinetics of the reaction of cytochrome c with $^{\bullet}CO_3^-$ was studied at 600 nm, the maximum of absorption spectrum of $^{\bullet}CO_3^-$.

Nitrogen dioxide ($^{\bullet}NO_2$) radicals were produced by the radiolysis of aqueous solution of 10 mM $NaNO_2$ in 10 mM phosphate buffer, saturated with N_2O (pH 7.25). The rate of the reaction of cytochrome c with $^{\bullet}NO_2$ was measured by competition with 0.025 mM 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) ($ABTS^{2-}$). The rate of formation of $ABTS^{\bullet-}$ was examined at 728 nm.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The decay of $^{\bullet}CO_3^-$ was accelerated in the presence of cytochrome c (Fig 1). The observed first order rate constants were linearly dependent on the protein concentration. Linear fit gave

second order rate constant for the reaction of cytochrome c with carbonate radicals of $(1\pm0.2)\cdot10^8$ $M^{-1}s^{-1}$ (pH 8.0).

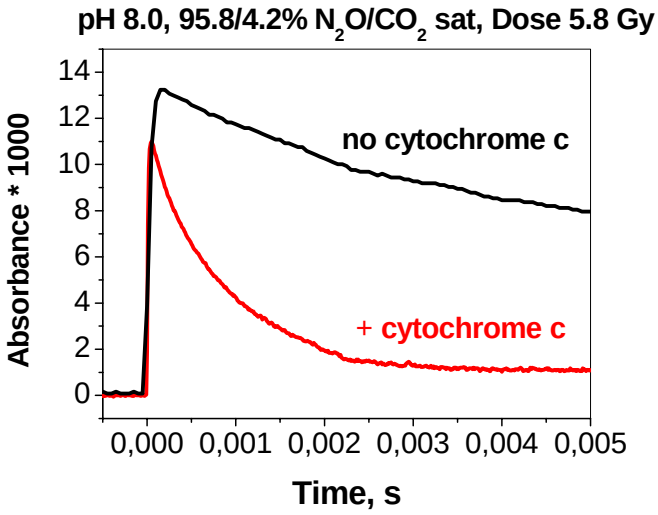

Fig.1. Kinetic traces of the decay of $CO_3^{\bullet-}$ at 600 nm in the absence and in the presence of 15 µM cytochrome c.

The rate of formation of $ABTS^{\bullet-}$ radical increased with increasing concentration of cytochrome c. On the other hand $ABTS^{\bullet-}$ was not stable in the presence of the protein and decayed over several milliseconds with the rate increasing with the concentration of cytochrome c (Fig. 2).

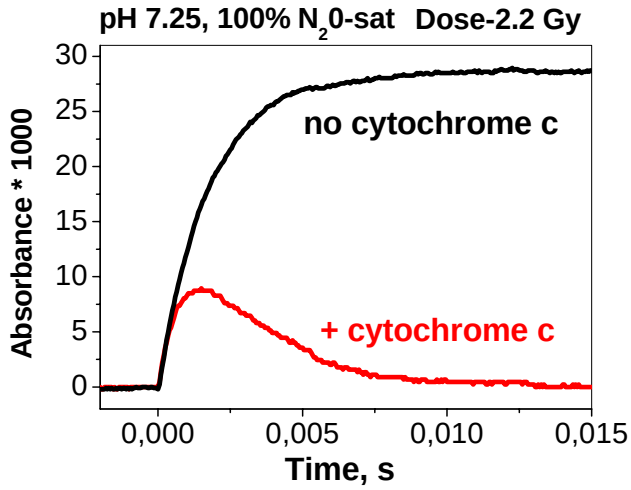

Fig 2. Changes of absorbance at 728 nm in the absence and presence of 60 µM cytochrome c.

Dependence of the first order rate constants for $ABTS^{\bullet-}$ formation on cytochrome c concentration gave second-order rate constant equals $(2.5\pm0.2)\cdot10^7$ $M^{-1}s^{-1}$.

FUTURE COLLABORATION WITH HOST INSTITUTIONS

It would be valuable to do more pulse radiolysis experiments in Gray Cancer Institute connected with the reactions of carbonate and nitrogen dioxide radicals with biomolecules and to discuss scientific results with Prof. Wardman and colleagues working in the host institution.

PROJECTED PUBLICATIONS/ARTICLES RESULTING OR TO RESULT FROM THE STMS

I am going to write the paper concerning the kinetics of the reactions of $CO_3^{\bullet-}$ and $^{\bullet}NO_2$ radicals with ferric cytochrome c very soon.

Joanna Didik, 12 March 2006

CONFIRMATION BY THE HOST INSTITUTE OF THE SUCCESSFUL EXECUTION OF THE MISSION

On behalf of the Gray Cancer Institute, I (Peter Wardman), confirm that Joanna Didik attended the Institute for two weeks in February 2006 as described above. She received training in the use of the pulse radiolysis facility and data analysis software. She complied with Institute regulations for health and safety and radiation protection. The data analysis software was also provided for her to install on a PC at her home institution for later analysis.
I was pleased that the experiments went much as anticipated and that she was able to obtain the key information desired. We would be pleased to cooperate in a similar manner again.

Signed (signature obtainable on request): Peter Wardman, 15 March 2006
Deputy Director, Gray Cancer Institute