Research Group for Bioactinide

Group Leader: Toshihiko Ohnuki

Our research subject is focused on the formation of actinides and lanthanides nano-particles in the biological reaction environments. The central objectives are to elucidate the biological and chemical processes of the nano-particles formation by analyzing change on chemical states of acitinides by advanced analytical methods. Among the interaction of actinides and lanthnides with microorganisms, nano-particle formation by the transfer of phosphorous ions and electron from the cells to actinides has been studied.

Formation of phosphate nano-particles in the biological reaction environments

Phosphate minerals containing heavy elements of lanthanides and U are known to be hardly soluble in the aqueous solution in the environments. Phosphorous is an essential element of microorganisms. The microorganisms store P in their cells. We have found that yeast, *Saccharomyces cerevisiae* releases P in solution containing U(VI), followed by the formation of uranyl phosphate mineral of H-autunite [1]. However, not only mechanism of the formation of uranyl phosphate mineral, but role of microorganisms for the formation have not been elucidated.

We have conducted the research of phosphate mineralization of one of the lanthanide elements of Ce on the cell surface of yeast [2]. The yeast cells were exposed to Ce containing solution. Cerium concentration in solutions decreases as a function of exposure time. Analyses of the yeast cells by FESEM, TEM, and XAFS show that needle-shaped Ce(III) phosphate nanocrystallites with a monazite structure formed on the yeast cells by exposure to Ce(III) for 42 h, even though the initial solutions did not contain any P species. The Ce(III) phosphate nanocrystals grew from about 50 nm to hundreds of nanometers. P concentration in the solution increased after the yeast cells were inoculated, indicating the release of P from the yeast cells. These results suggest that the sorbed Ce on the cell surfaces reacted with P released from inside the yeast cell, resulting in the formation of Ce(III) phosphate nanocrystallites.

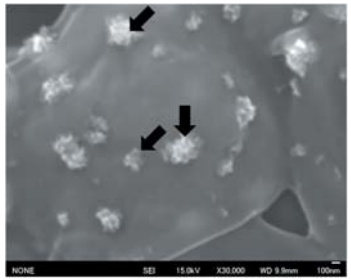

Fig. 1 SEM image the precipitates formed on the yeast cell surface after exposure to 1.79 × 10^{-4} mol·L^{-1} of Ce solution for 7 days at pH 5. Bright spots sized 100-200 nm with black arrow are observed on the cell.

Formation of nano-particles by electron transfer in the biological reaction environments

Electron is transferred when microorganisms respire to obtain energy. Some microorganisms, i.e. iron reducing bacteria, can reduce soluble U(VI) to insoluble U(IV) using organic substrates or hydrogen as electron donor. By the reduction of U(VI) to U(IV), nano-particles of UO_2 have been formed. However, mechanism of the formation of UO_2 nano particles have not been elucidated. In this year, we have examined the electron pathway for the U(VI) reduction mediated by flavin mononucleotide (FMN), which is secreted by *Shewanella* species [3].

The cyclic voltammetry (CV) and photo-electrochemical methods with an optically transparent thin-layer electrode (OTTLE) cell were utilized in investigating in vitro the electron transfer reactions that take place between FMN and U(VI). In the CV measurements of U(VI), a catalytic U(VI) reduction current was observed in the presence of FMN at the redox potential of the FMN. The reduction current increased with an increase in the concentration of the U(VI). The reduced form of the U was confirmed to be U(IV) by the photo-electrochemical analysis using the OTTLE cell. The results demonstrated that FMN acts as the mediator in the electro-reduction of U(VI) to U(IV). In addition, in-vivo bio-reduction experiments on U(VI) with *Shewanella putrefaciens* revealed that the addition of FMN accelerated the reduction rate of the U(VI). These results indicate that the bio-reduction of U(VI) by the *Shewanella* species can be catalyzed by FMN secreted from the cells.

Electron transfer from Mn(II) to manganese oxidizing microorganisms causes the formation of Mn(IV) oxides. Manganese oxides have oxidizing properties for Ce(III). The oxidation of Ce(III) by Mn oxides causes positive Ce adsorption anomaly in the rare earth elements (REE) [4]. We have found that the positive Ce anomaly for the biogenic Mn oxide detected at pH 3.83 decreases with increasing pH. Suppression of the positive Ce anomaly under higher pH is recognized for the biogenic Mn oxide, and further negative Ce anomaly is observed at a pH of more than 6.5 [5]. The trend observed for our data is quite opposite to that observed for the previous studies using abiotically synthesized Mn oxide, where the degree of positive Ce anomaly increases with increasing pH. The detail of the research results is shown in the research highlight.

References

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Research Group for Radiation and Biomolecular Science

Group Leader: Akinari Yokoya

The research objective of Radiation and Biomolecular Science Group is to fully characterize molecular processes by which ionizing radiation alters the chemical structure of biomolecules, particularly the genetic material (DNA), in cells. Two approaches are proposed to address the main objective: 1) Determine the physicochemical processes of DNA alteration induced by radiation in terms of electron/hole transfer within DNA and the hydrated water layer surrounding DNA. 2) Obtain experimental evidence of cellular responses to radiation, including DNA repair processes to various types of DNA alteration in living cells.

Study of unpaired electron species in pyrimidine DNA-bases induced by nitrogen and oxygen *K*-shell photoabsorption

In order to clarify the mechanism of DNA-base modification (base lesion) induced by *K*-shell photoabsorption of nitrogen and oxygen atoms, we have developed an X-band electron paramagnetic resonance (EPR) spectrometer system at a synchrotron soft X-ray beamline BL23SU in SPring-8, and examined the EPR '*in situ*' signal during the soft X-ray irradiation. Spectra of two DNA pyrimidine bases, thymine and cytosine, were measured and their fine structures around nitrogen and oxygen *K*-edges were observed. The *g*-factor of the short-lived unpaired electron arising only during irradiation in thymine was determined to be 2.000, which is distinct from that of a free electron (2.0023). The EPR intensities for cytosine are significantly enhanced by nitrogen (Fig. 1) and oxygen *K*-absorption, indicating that cytosine favors to form unpaired electron species, rather than thymine, presumably due to the excitation of the enhanced electron capturing [1].

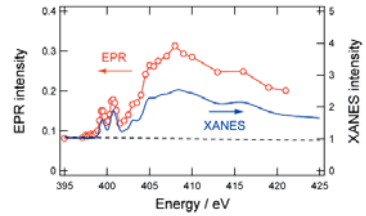

Fig. 1 Dependence of the EPR intensity of cytosine film on soft X-ray energy around nitrogen *K*-edge. The right-hand axis shows the intensity of the X-ray absorption near edge structure (XANES) spectrum. The dotted line represents the baseline deduced from the calculation of the photoabsorption cross section of cytosine assuming that the cross section is determined by summing up each elements cross sections, without nitrogen *K*-shell.

The significance of a base modification with a GAP type strand break within a clustered DNA damage site on the induction of mutations

Base lesion(s) induced by high LET radiation associated with other types of damage, known as a clustered DNA damage site, compromise(s) base excision repair proteins [2]. The effect of clustered DNA damage sites *in vivo* still remains largely unknown. We examined whether two- or three-lesion clustered damage site containing an 8-oxoG(s) and a single strand break (SSB) with hydroxyl groups at the both strand-break termini (called "GAP" type SSB) is highly mutagenic, using plasmid based assay in *Escherichia coli (E.coli)* to musure mutation frequency of these clsutered DNA damage sites [3]. The experimentaly determined mutation frequencies of bi-stranded two-lesion clusters (GAP/8-oxoG), especially in *mutY* deficient *Escherichia coli* strain, are high or are similar to those for bi-stranded clusters with 8-oxoG and other base modifications, such as dihydrothymine, or abasic site (AP site), suggesting that the GAP is processed at similar efficiencies with 8-oxoG or AP site within a cluster. The mutation frequencies of tandem two-lesion clusters comprised of an 8-oxoG and a GAP are, on the other hand, comparable to or less than that of single 8-oxoG. Mutagenic potential of three lesion clusters, which were comprised of a tandem lesion (an 8-oxoG and a GAP) and an opposing single 8-oxoG, was higher than that of a single 8-oxoG but was no more than that of a bi-stranded 8-oxoGs. We suggest that although the nucleotide incorporation opposite 8-oxoG plays an important role, it is less, at least in part, mutagenic when GAP is present on the opposite strand. Our observations indicate that the repair of a GAP is retarded by opposing, but not by tandem, 8-oxoG and that the extent of GAP repair determines the biological consequences.

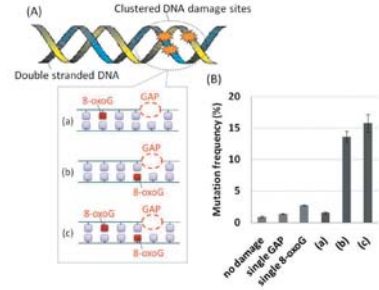

Fig. 2 Mutation frequencies of two- or three- lesion clustered damage sites containing 8-oxoG(s) and a GAP transformed into *mutY* deficient strain of *E.coli*. (A), scheme of three types of clustered damage sites on double stranded DNA. (B), mutation frequencies of these clustered damage sites transformed into *mutY* deficient strain of *E.coli*.

References

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