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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Reduction of Cr(VI) as a result of interaction with lipid membrane studied by XAS.	**Experiment number**: CH-5210
Beamline: BM25A	**Date of experiment**: from: 21.02.2018 to: 24.02.2018	**Date of report**: 04.11.2018
Shifts: 9	**Local contact(s)**: Dr. Aida Serrano	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Aim of the experiment

The main goal of the experiment was to study products of interaction of Cr(VI) in form of $K_2Cr_2O_7$ into lipid bilayers composed of either pure 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) or mixed with 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). Lipid bilayers which contained DOPE had certain amount of double C=C bonds in alyphatic chains. Using Proton Induced X-Ray Emission (PIXE) technique, we have already shown that when increasing amount of DOPE in membrane, more Cr is incorporated into this membrane after interaction with Cr(VI). Additionally, in literature there is strong evidence that Cr(VI) has large permeability ratio throughout lipid membranes and during interaction it reduces into Cr(III), although direct mechanism of this process is unknown.

Experimental

Samples were prepaed in form of liposomal suspensions and solid pellets. Liposomes composed of DMPC/DOPE in ratios of 100:1, 10:1, 5:1 and 1:1 as well as of pure DMPC were treated with 150 mM of $K_2Cr_2O_7$ for 15 min in room temperature (RT). All samples were further dialysed in 55 °C for 19 h, to get rid of unbound Cr content. Half of samples were dried afterwards. Liquid samples were measured in RT in large volume, only in X-Ray Absorption Near Edge Spectroscopy (XANES) region due to low concentration of samples, while solid samples were mesured in 10 K and in RT (to study any radiation damage) in XANES and Extended X-Ray Absorption Fine Structure (EXAFS) regions. Beam spot size was between 4 x 1.5 mm^2 (V x H) and 2 x 5 mm^2, while beam spot was little out of focus in order to reduce radiation damage. X-Ray fluorescence was detected by 13-element HPGe Sirius (Sensortech) detector.

Results

Differences in near-edge spectra were observed for all samples however for liquids, changes in spectra were discreet and all spectra have shown pronounced pre-edge peak characteristic for Cr(VI) compounds. Additionally edge shifted around 2 eV from DMPC +Cr to DMPC/DOPE (1:1) +Cr.

More pronounced differences were seen in spectra obtained from pellet measurement both in XANES and EXAFS regions. In the near -edge energy range it can be clearly seen that while sample contained more DOPE, the edge position was shifted more into low energy region. The total edge shift between $K_2Cr_2O_7$ and sample DMPC/DOPE (1:1) + Cr is roughly 5 eV which corresponds to reduction of oxidation state from +6 to +3 (Fig. 1 A). EXAFS signal has good statistics up to k equal to 11-12 $Å^{-1}$ (Fig. 1 C). It is clearly seen that, coordination environment around Cr atom significantly changed : while for DMPC and DMPC/DOPE

(1:100) EXAFS signals are very similar to $K_2Cr_2O_7$, the DMPC/DOPE (10:1) and (5:1) are similar to each other and completely different to reference. Comparison of this conclusion with XANES spectra on Fig. 1 A, shown it was becouse spectra of DMPC/DOPE (10:1) and (5:1) were combination of $K_2Cr_2O_7$ and reduced form of Cr which was the most abundant in the DMPC/DOPE (1:1) + Cr sample. The LCF proved this statement and results are summarized in Table 1 and in Figs. 1 D-E. The progressing reduction is clearly visible also in the Fig. 1 B, where changes in pre-edge peak characteristics as well as in edge position are shown and are behaving in similar way to described above. The last spectrum of DMPC/DOPE (1:1) + Cr sample is for the most reduced Cr. Moreover there is no trace of Cr(VI) and this spectrum is completely different than any other. XANES spectrum of DMPC/DOPE (1:1) + Cr sample is however similar to some Cr XANES spectra reported for bacteria cells treated with Cr(VI), yet spectrum was not clearly identified [1]. Simultaneously, the only thing that was effectively varying in our experiment was amount of C=C bonds, thus we can rule out possibility of dominating factor of Cr binding to phispholipid heads. The double pre-edge peak for this spectrum indicates different ligands in 1st coordiantion shell of Cr. Our initial FEFF calculations on model Cr-lipid structures have shown that each those maxima can originate from C – pDOS, O-pDOS contributions mixed with Cr d-DOS. Taking all together, this is a proof, that obtained results present increasing content of organochromium complexes.

	K2Cr2O7	DMPC/DOPE (1:1) + Cr
DMPC/DOPE (10:1) + Cr	0.565(8)	0.435(26)
DMPC/DOPE (5:1) + Cr	0.511(7)	0.489(25)

Table 1 LCF results for DMPC/DOPE (10:1) and (5:1) + Cr samples.

[1] D. Long, L. Zou, M.Z. Hashmi, K. Cai, X. Tang, G. Chen, and J. Shi, Chem. Eng. J. **280**, 763 (2015).

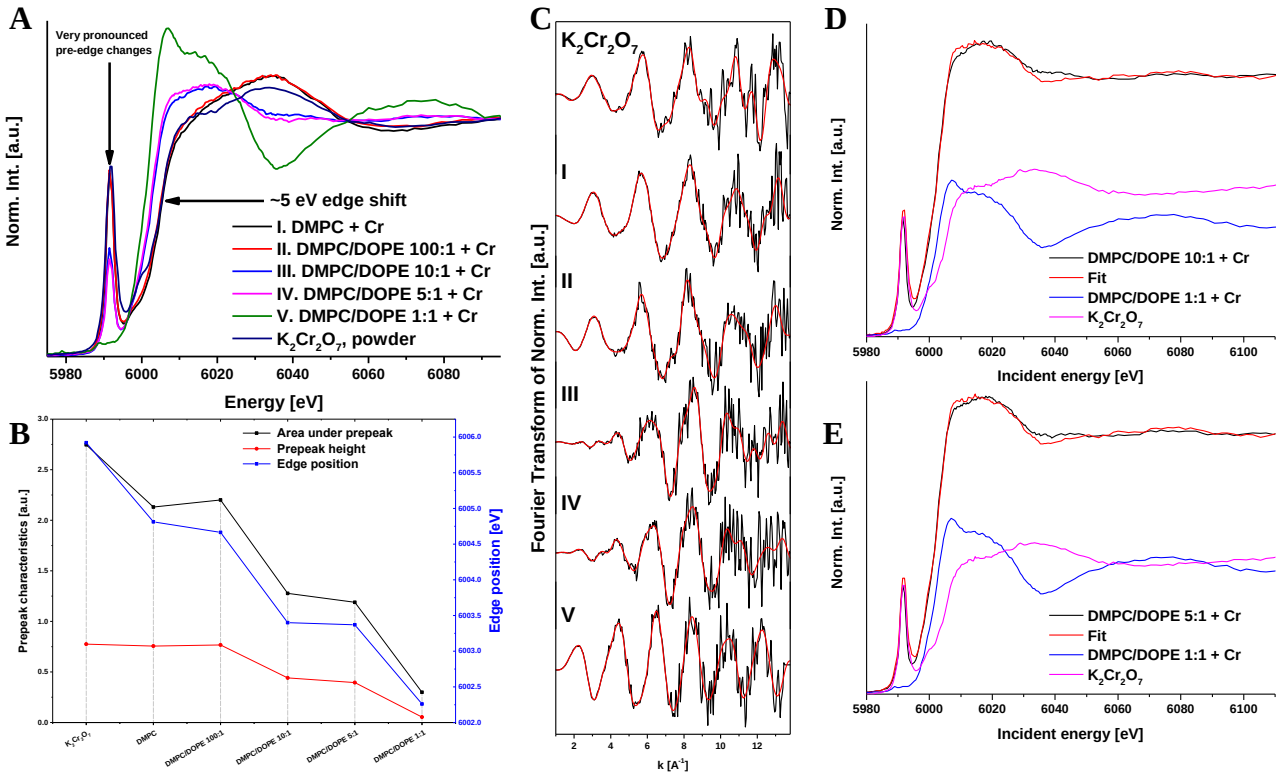

Fig. 1. A –XANES spectra of liposomal samples and $K_2Cr_2O_7$ reference; B –Changes in the structure of pre-edge peak and edge position; **B** – EXAFS signal for liposomal samples and $K_2Cr_2O_7$ reference: Fourier Transform (black) and Backward Fourier Transform (red); **C** – EXAFS signal for liposomal samples and $K_2Cr_2O_7$ reference: Fourier Transform (black) and Backward Fourier Transform (red); **D** – DMPC/DOPE (10:1) + Cr sample fitted with $K_2Cr_2O_7$ reference and the most reduced DMPC/DOPE (1:1) + Cr sample; **E** – DMPC/DOPE (5:1) + Cr sample fitted with $K_2Cr_2O_7$ reference and the most reduced DMPC/DOPE (1:1) + Cr sample.