EXPERIMENTAL REPORT FOR PROPOSAL MD-1028

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for probing amyloid related biological issues.
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Background Summary :

Zinc (Zn) is one of the most common metal elements in the human body and several studies highlighted its importance for the male reproductive physiology. Hence, Zn concentration in seminal plasma is considerably higher than that in blood and appears to influence key functions that support spermatozoa fertilization potential like motility, activation (capacitation, acrosome reaction) and chromatin decondensation. Previous works have analyzed the availability of Zn^{2+} in seminal plasma and revealed that the latter is not a simple solution of metal but a highly dynamic system characterized by exchanges between several pools. Briefly, during the sequence ejaculation-coagulation-liquefaction of the semen, Zn is supposed to be sequentially transferred from citrate to high molecular weight proteins and then to low molecular weight proteins (LMWP). Such transfers would modify Zn^{2+} availability for ejaculated spermatozoa and impact their functional competence. However, the biochemical mechanisms involved in such transfers have not been deciphered yet.

Objective and strategy :

Our goal was to characterize the different Zn^{2+} pools through XANES and EXAFS exploration of carefully chosen and comprehensively analyzed seminal plasma samples. We mainly explored the following samples: two pools of normal seminal plasma which both exhibit normal Zn, citrate and LMWP concentrations and a pool of seminal plasma from obstructive azoospermic patients which harbors an abnormal composition of LMWP.

Composition of seminal pools used in the experiment :

	Citrate (mmol/L)	Zn (mmol/L)	pH	Peptides (g/L)	Standard LMWP (%)
Pool PS 2015	25.83	1.8	7.7	28.32	~100%
Pool PS 2016	24.42	2.67	7.8	29.99	~100%
Azoo Pool 2017	94	9	6.8	39	0%

Experiments details :

Beamline	Round Beam	Time allocated	Local Contact
BM26A (C04) DUBBLE	10/2016	9 shifts	Dr BANERJEE

Main Results :

1- Zn signature of human seminal plasma (Figure 1) :

To the best of our knowledge, we report here the first Zn XANES spectra of human seminal plasma. We used the spectra of the mix Zn + Citrate as reference : it exhibits a high and narrow peak around 9660 eV that indicates a high level of coordination of Zn and a specific shape between 9670 and 9750 eV with two couples hump-valley. The spectra of seminal plasma from azoospermic patients (Azoo) exhibited the same shape with a slightly lower peak at 9660 eV. Thus, in absence of LMWP, seminal Zn is mainly coordinated by citrate. In normal seminal matrixes (Pool 2015 and 2016), the peak is significantly lower and the shape is different with a disappearance of the first couple of valley-hump. This indicates that Zn coordination has been modified by the presence of LMWP. Of interest, the progressive mixing of Azoo plasma with normal

plasma modifies the shape of the spectra in a way that could be explained by a transfer of ZN from citrate to LMWP, confirming previous studies (data not shown)

2- Impact of Zn overload on seminal plasma XANES spectra (Figure 2) :

We overloaded normal seminal plasma with Zn at 20mM and hypothesized that the Zn in excess will coordinate back to citrate. It was not the case since the overall shape of the spectra was smoother than that of the normal matrix. This indicates that seminal LMWP were able to link a ten-fold excess of Zn.

3- Change of seminal Zn signature by L2 chelatant :

L2 chelatant is specific for Zn and is able to modify the shape of Zn sulfate and Zn citrate spectra by smoothing the first couple hump-valley (Figure 3). L2 affinity for Zn is thus higher than that of both anions. Mixing the biological matrixes with L2 results in the same shape for each spectra confirming that L2 has a higher affinity than both citrate in vivo and LMWP (Figure 4). L2 is then a suitable tool to manipulate Zn affinity for biological ligands in further experiments.

Conclusion :

This experiment provided us the first XANES signature of seminal Zin, it confirmed that Zn can be coordinated in vivo by citrate and that the order of affinity for Zn is the following : citrate < LMWP < L2. These results open the way to identify which LMWP are involved in seminal Zn coordination and to decipher the metal enviroment around spermatozoa.

Figures :

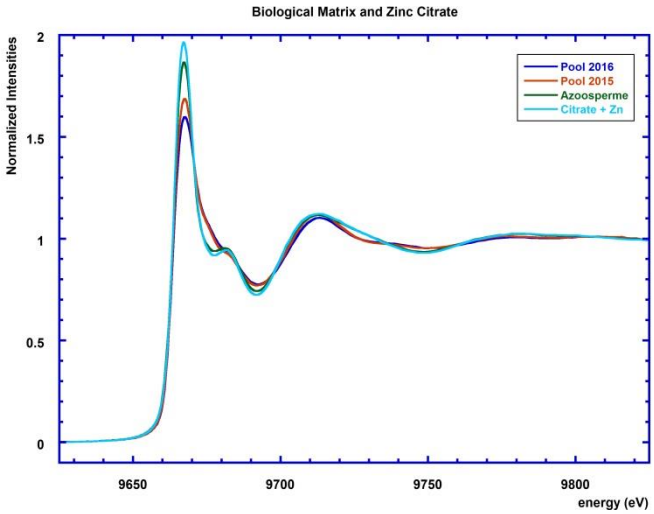

Figure 1

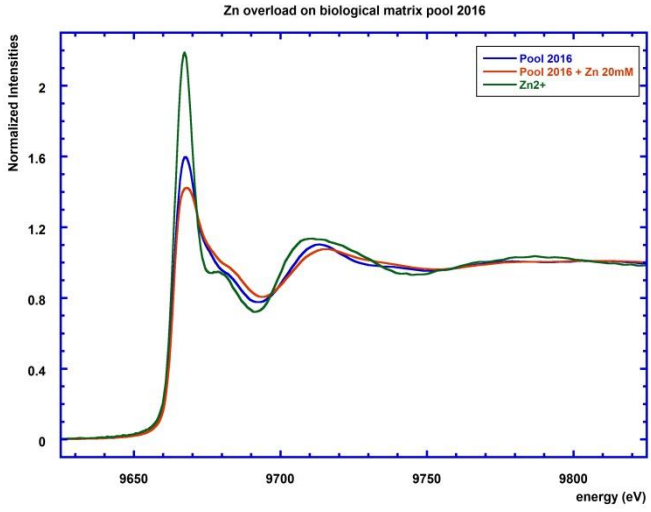

Figure 2

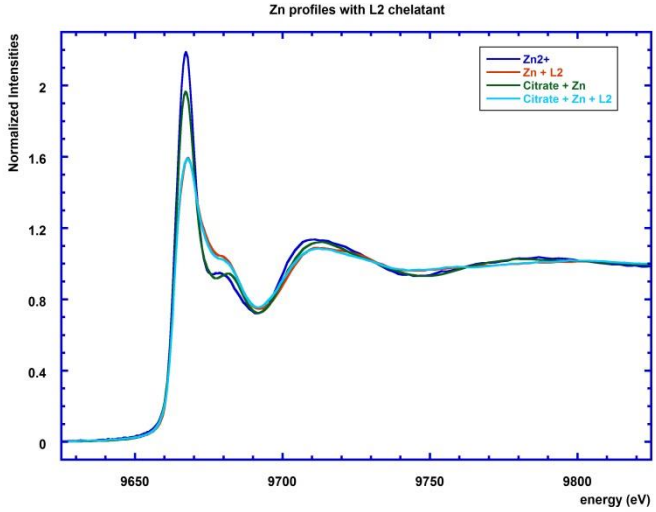

Figure 3

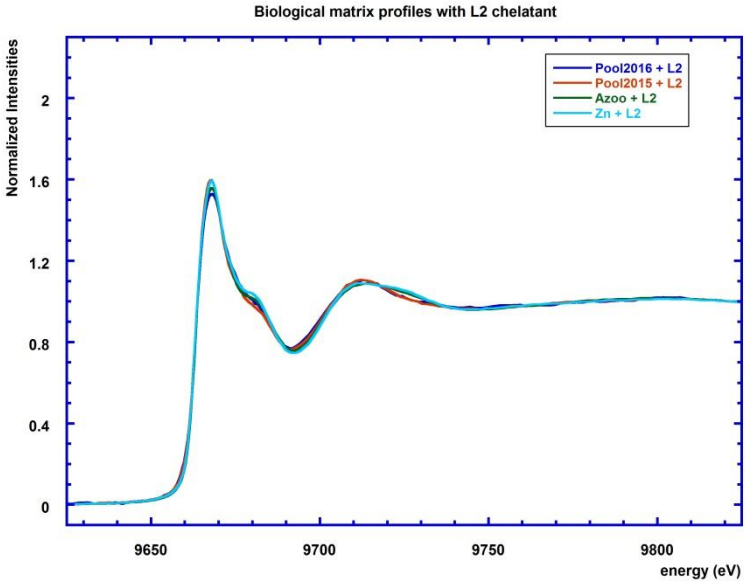

Figure 4