



	Experiment title: EXAFS Study of Micellar Aggregates of Bile Calcium Salts	Experiment number: CH-963
Beamline: ID26	Date of experiment: from: 25/10/2001 to: 30/10/2001	Date of report: 21/09/2002
Shifts: 15	Local contact: Pierre-Emmanuel PETIT	<i>Received at ESRF:</i>
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Report:

The local coordination of Ca^{2+} in water and in aqueous micellar aggregates of bile acid calcium salts has been studied with the EXAFS technique.

Bile salts and their conjugated derivatives can be considered one of the most important class of natural detergents. In aqueous solution they give rise to micellar aggregates and interact with several biological compounds such as cholesterol, bilirubin-IXa, phospholipids, glycerides and fatty acids. They are present in the bile, where they are involved in many regulatory mechanisms, and they play a pivotal role in the emulsifying process of the dietary lipids in the small intestine. Due to their solubilization properties, many efforts have been made to determine the micellar structure of bile salts.

Calcium plays an important role in the biliary milieu. In bile calcium ions interact with various anions, including those of bile salts, reducing their activity. Physiological complexes of calcium ions with bile salts are present both in hepatic and gallbladder bile. The nature of the interactions between calcium ions and bile salt anions and the effect of the competition between calcium and sodium ions in the formation of micellar aggregates, could explain the precipitation mechanism of insoluble calcium salts. These nuclei determine the initiation and growth of pigment gallstones.

Studies on calcium ion binding to deoxycholate, taurodeoxycholate (TDC) and glicodeoxycholate (GDC) have been recently carried out in our laboratory with different techniques such as X-ray diffraction, quasi-elastic light-scattering (QELS) and CD. The QELS measurements show that Ca^{2+} ions have a bigger affinity for the bile salt anions than Na^+ ions. Micellar aggregates with Na^+ and Ca^{2+} ions seem to be more stable than those containing Na^+ ions only. The addition of Ca^{2+} ions to bile NaTDC and to NaGDC micellar solutions induces a sudden increase of the hydrodynamic radii. In particular, the

taurodeoxycholate micellar size increases much more with CaCl_2 than with an amount of NaCl corresponding approximately to the same ionic strength. It is probable that the Ca^{2+} ions substitute the Na^+ ions and join the NaTDC micellar aggregates forming aggregates of bigger size.

The EXAFS spectra of Ca^{2+} ion in aqueous and acetonitrile solutions, together with several calcium and calcium–sodium bile salts solutions have been collected.

All spectra show evident contributions of strong multielectron excitations in the range 25–60 eV. It is well known that in the case of solutions, it is important to analyse the EXAFS spectra at low k values, in order to maximize the spatial resolution, to better analyze asymmetric distributions and to detect the presence of multiple scattering contributions associated with triangular correlation functions. An accurate determination of the structural parameters is possible only if double–electron channels are accounted for. The exclusion of these effects results in systematic errors on structural parameters.

A method to analyze EXAFS spectra of liquid systems, starting from two– and three–body correlation functions obtained from molecular dynamics simulations (MD) has been developed by our group [1]. MD correlation functions are used as relevant models in the calculation of the EXAFS structural signal of ions in solution. Removal of the EXAFS structural contributions from the absorption spectra allow the isolation of multielectron transition features and the identification of their positions.

MD simulations of Ca^{2+} in water have been performed and the Ca–O and Ca–H two–body asymmetric radial distribution functions and the O–Ca–O first hydration shell three–body correlations have been determined. The structural signal of Ca^{2+} ion in aqueous solution has been calculated and the parameters describing the correlation functions have been refined by fitting the theoretical signal to the raw experimental data. The analysis has been performed using the GNXAS code, in a k range from 3.7 and 10.5 \AA^{-1} .

In a second step, the analysis has been performed including the low k region ($k > 2.6 \text{\AA}^{-1}$). The refined structural parameters have been used to calculate a total theoretical signal. A comparison with the experimental spectrum shows in the residual curve the presence of features which are associated with the opening of double–electron excitation channels. These resonances are associated with the $1s3p$ and $1s3s$ double–excitation channels. The shape and the intensity of the double–excitation features have been obtained from the fitting procedure. The energy positions correspond to the predictions of the $Z+1$ approximation [2].

The EXAFS analysis of the bile salt solutions containing calcium has been performed, including the multielectron excitations in the fitting procedure. No peculiar calcium coordination has been observed: the coordination is very similar to that found for the hydrated calcium.

1) P. D'Angelo and N. V. Pavel, *J. Synchrotron Rad.* 8, 173 (2001) and references cited herein.

2) P. D'Angelo, P. E. Petit and N. V. Pavel, manuscript in preparation.