When the Synchrotron radiations highlight the Randall’s plaques and kidney concretions

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Abstract. In western countries, a dramatic increase in papilla calcifications (Randall’s Plaque or RP) is observed as a major cause of calcium oxalate kidney stones. Through ex vivo X-ray absorption spectroscopy, we give for the first time direct structural evidence of the presence of amorphous carbonated calcium phosphate in these Randall’s plaques (RP). Such chemical composition of RP present in increasingly young subjects raises a major question regarding alimentation: does nutrient-enriched food especially aimed at young children affect the physiology of the kidney? Moreover, lithogenic diseases may induce intratubular crystallization and end-stage renal failure. We show that Fourier transform infrared microspectroscopy is able to characterize such pathological microcalcifications giving their chemical composition and their spatial distribution, thus providing invaluable information for the diagnosis of the disease and the treatment of the patients.

1. Introduction

Pathological calcifications present are associated with major public health problems such as type 2 diabetes [1,2,3]. For several years, we have conducted a translational research defined through a multiscale description of their structural and chemical characteristics in order to propose physical methods as diagnostic tools [4], to establish a relationship between their structural characteristics and medical management [5], or to describe the biochemical processes which lead to their pathogenesis [6]. This research program encompasses the investigation of pathological calcifications present in kidney [7], prostate [8], cartilage [9], cardiac valves [10] and thyroid. Synchrotron radiation (SR) related techniques play a key role in our research [11-16]. Indeed, the very first spectra collected on Soleil were collected on kidney stones (KS) [17].

We have investigated a calcified plaque named Randall’s Plaque (RP) deposited beneath the epithelium near the tip of renal papillae [6]. Such plaques act as an anchored nidus for Ca oxalate (CaOx) stones which exhibit a concave region (figure 1). A dramatic increase in the prevalence of KS developed from a RP was observed in western countries. As we can see in figure 1, in 2010, more than 55% of CaOx KS display a RP at their surface in young stone formers aged 20 to 30 years instead of 16% in the nineties. We have characterized RP through X-Ray absorption spectroscopy (XAS) at the Ca K edge. XAS is able to describe accurately the electronic state, the geometry of the very first...
neighbors and finally the first coordination spheres of a selected element [18,19]. Such ex vivo configuration preserves the state of crystallization which depends on the hydration level.

![Image](https://example.com/image.png)

Figure 1. Left: CaOx monohydrate stone nucleated from a papillary RP (arrow). Right: Increase with time of the proportion of calcium oxalate stones nucleated from a RP

We have also assessed the chemical diversity of ectopic calcifications present in kidney tissue through SR–Fourier transform infrared microspectroscopy (μSR-FTIR) experiments. Such approach provides an accurate description of the different chemical phases present in tissue leading to an understanding of the kidney function loss.

2. Material and methods
The selected samples were investigated either on the DIFFABS beamline situated at the D13-1 bending magnet or on the SMIS beamline at SOLEIL-synchrotron [16,17,20,21]. All the selected samples come from Necker Hospital. Regarding the FTIR experiments, five microns slices of the biopsies were deposited on low-e microscope slides (MirrIR, Kevel Technologies, Tienta Sciences, Indianapolis). For tissue embedded in paraffin, the paraffin was chemically removed. The compounds were identified by comparing them to reference spectra [22].

3. Results and discussion
At either the K- or the L-edge, XAS contains both electronic and structural information [23,24]. To overcome such difficulty, we consider chemical compounds with well-known atomic structure. In figure 2, we have plotted three reference compounds namely hydroxyapatite (HAP), a kidney stone made of calcium phosphate apatite (CA) as well as a kidney stone made of amorphous carbonated calcium phosphate (ACCP). Due to the presence of the calcification at the top of the papilla, the amplitude of the absorption edge (from 1a to 1d in figure 2) and thus the Ca content decrease rapidly at lower acquisition points on the sample. The fact that the shoulder B is absent along with a single feature instead of C1 and C2 structures demonstrate that the Ca phosphate compound is close to ACCP [25]. We give thus for the first time direct structural evidence of the significant presence of ACCP as a major constituent of RP [26]. Moreover, our measurements suggest that ACCP are deposited within the tissue, and not only at the surface of the papilla. From a biochemical point of view, it is worth to underline that in kidney ACCP is evidence of an oversaturation in Ca phosphate by an excess of Ca and/or phosphate and/or due to a too high pH. Such chemical composition of RP present in increasingly young subjects raises a major question regarding alimentation: does nutrient-enriched food specially aimed at young children affect the physiology of the kidney? Since X-ray absorption near edge structure (XANES) is insensitive to polydispersity [27], it is possible that other Ca phosphates are present as minor phases as well.
Figure 2. Left: XANES part of the absorption spectra for the papilla 1 and reference compounds. Right: The RP can be clearly seen as the white part at the top of the renal papilla. a, b, c and d refer to acquisition points related to X-ray absorption experiments.

Among twenty four kidney biopsies from patients suffering severe chronic kidney disease and, in some cases (n=8), end stage renal failure [19], we found an unexpected variety of compounds, most of them being probably involved in the worsening of kidney function. To our best knowledge, many of these compounds were never reported in crystal deposits within the kidney (table 1).

Table 1. Crystalline phases identified in kidney biopsies of patients suffering severe chronic kidney disease

| Chemical compounds     | Crystalline phases                                      |
|------------------------|---------------------------------------------------------|
| Calcium oxalate        | Calcium oxalate monohydrate, calcium oxalate dihydrate |
| Calcium phosphate      | CA, ACCP*, Octacalcium phosphate pentahydrate*, whitlockite* |
| Calcium carbonate      | Calcite                                                 |
| Purines                | monosodium hydrogen urate monohydrate*, methyl-1-uric acid*, 2,8-dihydroxyadenine |
| Silicium derivatives   | Amorphous silica*                                       |
| Drugs                  | Sodium foscarnet                                        |

*components not previously reported in kidney biopsies
Their clinical significance often remains unclear. However, most among these compounds were previously identified in urinary calculi and related to specific clinical pathologies. For example, it is well known that inherited diseases such as adenine phosphoribosyltransferase deficiency commonly are responsible for kidney stone disease as a result of very low solubility of the dihydroxyadenine. However, in some patients, no stone is formed despite a permanent urine crystallization and the kidney function may progressively deteriorate because extensive deposits of crystals within the kidney. Thus the disease is often late diagnosed, even after a kidney graft. In other cases, the mechanism involved in kidney failure is expected such as in the case of drug therapy with foscarnet in patients affected by a cytomegalovirus infection. It was a long time ago reported that the drug was able to crystallize within glomeruli of the kidney, thus leading to renal failure. However, thanks to Synchrotron FTIR microscopy, we were able to demonstrate that, in addition to glomerular deposits of the unchanged drug, its metabolites, mainly as calcium apatite salt, may crystallize within tubule cells, thus worsening the kidney dysfunction. Lastly, for other crystals identified in the biopsies, the cause of their formation remains to be identified. Chronic exposure to potentially toxic environments such as excessive intake of aluminum or silicium derivatives could be involved at the origin of several phases such as methyl-1-uric acid or amorphous silica.

4. Conclusion

Fourier transform infrared microspectroscopy is able to characterize pathological calcifications giving their chemical composition and spatial distribution. SR techniques provide invaluable structural and chemical information at the cellular level and play now a pivotal role in medical diagnosis for some inherited diseases.

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