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Imaging of iodine and calcium in a section of rat thyroid using an x-ray microprobe

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Spectromicroscopy in the hard x-ray region has been realized using an energy tunable x-ray microprobe [1,2], and trace characterization using micro x-ray fluorescence (XRF) analysis has been carried out in this subject. The samples investigated are synthetic diamonds, tissue sections of biological samples and environmental aerosol samples. Advantage of the x-ray microprobe in the field of medical and biological fields is its capabilities for obtaining trace elemental distribution from conventional formalin fixed paraffin enbedded tissue sections without any pretreatment.

Fig. 1 shows calcium Kα and iodine Lα XRF images obtained from a thin section of a rat thyroid. Strong XRF signals were obtained in the dark pixels in the image, and the signal is proportional to the concentration of calcium and iodine in the section. A comparison between these two images shows clear absence of iodine in the parathyroid marked with the arrow in the figure. Moreover, iodine signal is independent from that of calcium even within the thyroid.

Fig. 2 shows an iodine XRF image of the sample with the higher magnification. The region is indicated in Fig. 1a). Difference of iodine XRF signals between individual follicles may be originated from the difference of thyroid hormone stored inside the follicle epithelium. The results suggests that the XRF imaging technique can be a powerful tool for diagnosis of individual cells and their activities.

References
1) S. Hayakawa et al., J. Synchrotron Rad. 8, 328 (2001).
2) S. Hayakawa et al., Anal. Sci. 17s, i115 (2001).

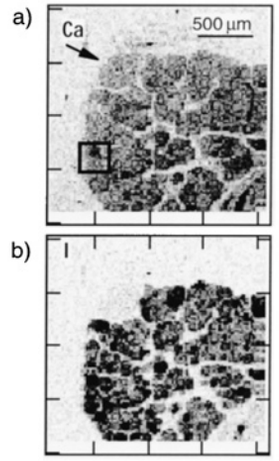

Fig. 1 a) Ca and b) I XRF images of a rat thyroid measured with 15 keV x-ray microbeam.

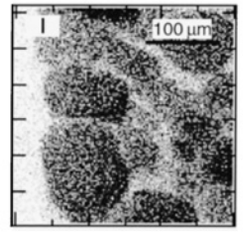

Fig.2 I XRF image of the rat thyroid with the higher magnification..

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Crystallographic data for phospholipase A_2, sucrose isomerase and α-amylase

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Even after several phospholipase A_2 (PLA2) structures are known, the structural studies of PLA2 continue to be interesting. PLA2s have sequence homology ranging from 40 to 99% and they have similar secondary and tertiary folding. Despite these similarities, they exhibit different pharmacological activities, like, myotoxicity, anti-platelet property and cardiotoxicity. We are mainly interested in the group-1B PLA2, which differs from the Group-1A by the presence of a pancreatic loop. Previously, all identified PLA2s in Group-1B are non-toxic, however, recently some toxic Group -1B PLA2 are identified. Therefore, it will be interesting to see the structural changes that mark a non-toxic protein to be toxic. In this proposal, we work on the crystal structure of one of the four phospholipase A_2 isoenzymes from *Micropechis ikaheka* (MiPLA3). MiPLA3 crystallized in a $P4_122$ space group with the unit-cell dimensions of a = b = 55.24, c = 146.3 . Unfortunately, our crystals diffracted at Spring8 only up to 4 . We are currently working on the structure determination and planning to improve the crystals.

Isomaltulose (α-D-glucosylpyranosyl-1.6-D-fructofranose), a sucrose with physical and organoleptic properties similar to those of sucrose, has now been suggested as a noncariogenic alternative to sucrose and widely used as a sugar substitute in food industry. Isomaltulose synthase, also known as sucrose isomerase, catalyzes the hydrolysis and isomerization of sucrose to produce isomaltulose, trehalulose (α-D-glucosylpyranosyl-1.1-D-fructofranose), and trace amounts of glucose and fructose as by-products. To further understand its mechanism of catalysis, we overexpressed, purified and crystallized isomaltulose synthase from Klebsilla sp. LX3 (KIS), which is a new bacterial isolate. Crystal was flash-cooled in liquid nitrogen and diffracted X-rays to 2.2 at beamline BL40B2, Spring8, Japan. Analysis of the diffraction pattern has shown that the crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 59.239, b = 94.153, c = 111.294 . The overall completeness (%) and R_{sym} (%) are 99.2 and 5.3 respectively. The asymmetric unit contains one enzyme molecule with the V_M of 2.31 ^{3} Da^{-1} and solvent content of 46.34 %. Currently, the structure determination and model building are underway. Enzymes with high efficiency and stability characteristics at higher temperature and salt concentrations, applicable for industrial processes, have high commercial values. α-amylase from the thermophilic halophile *Halothermothrix orenii* (AmyA) is a 55 KDa protein which cleaves starch, maltose and a few other sugars. It is stable and active significantly, at 338 K and in starch solution containing NaCl (up to 25% w/v). Structures of few thermophilic amylases have been studied. Therefore, solving the structure of AmyA will reveal what structural features make it both halophile and thermophile. Purified recombinant *AmyA* protein was crystallized by using hanging drop method. The crystal diffracted x-rays up to 1.89 at beamline BL40B2, Spring8, Japan. Analysis of the diffraction data shown that the crystal posses orthorhombic space group $P2_12_12_1$ with unit-cell parameters a = 55.126, b = 61.658 and c = 147.625 . A total of 85,503 reflections were collected in 180 frames, of which 38,334 are unique reflections with a redundancy of 2.2., completeness (%) 95.1 and the R_{sym} (%) 2.7. Currently the structure determination is underway.