In vivo monitoring of the growth of medaka eggs by near-infrared imaging

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Summary: The present study show the in vivo NIR imaging of the growth of fertilized eggs of Japanese medaka at the molecular level. The differences of material variations of protein and lipid and distributions with different water structures can be visualized. Furthermore, the detailed structure of embryo was also described by the distribution of different water structure combined with the usage of chemometric method. It is likely that life events occurring within the egg during development were shown to have an impact on the hydrogen bonding of water.

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Introduction: The monitoring of the eggs growth and eggs quality are important for aquaculture industry and infertility treatment. The evaluation method of egg quality is generally based on the morphological feature, but this method needs expert skills. Furthermore, the material variations within the egg such as DNA, proteins, and lipids during the egg growth are mainly investigated by the destructive methods. In this study, the growth processes of medaka eggs were analyzed non-destructively at the molecular level especially about the water structure using near-infrared (NIR) spectroscopy and imaging.

Methods: In the present study, the eggs of Japanese medaka (*Oryzias latipes*) were used from the first day after fertilization to the day just before hatching (JBH). Additionally, unfertilized, frozen-thawed, and the ones incubated at low temperature were also analyzed as samples of abnormal egg. Figure 1 is an optical image showing a fertilized egg on the 1st day after fertilization. Soon after the fertilization, the eggs become to consist of inhomogeneous structure. Many small oil droplets distributed throughout the egg begin to coalesce and fuse into larger droplets. The cytoplasm at the same time begins to gather to form a blastodisc which transforms into embryonic body in the future.

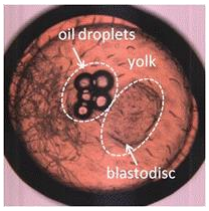

Figure 1: An optical image of a fertilized medaka egg on the 1st day after fertilization.

NIR measurements were performed using two instruments: one was a Perkin Elmer (Waltham, MA) imaging system consisting of a Spectrum One FT-NIR spectrometer coupled to a Spectrum Spotlight 300 NIR microscope, and the other was a microscope NIR imaging system with hyper spectral camera called Compovision (Sumitomo Electric Industries Ltd).

Results and discussion: In the NIR spectra, water, protein, lipid bands were observed. When the principal component analysis was performed to the data set including normal eggs on the first day after fertilization and abnormal eggs (unfertilized, frozen-thawed, and incubated at low temperature), they are classified into two groups by the band feature in combination mode of OH. Figure 2(a) and (b) demonstrate the score plots and loading plots of PC1 and PC3, respectively. It can be seen that normal eggs are separated from abnormal eggs. Figure 3(a) shows the second derivative intensity of weakly hydrogen-bonded (5282 cm^{-1}) and strongly hydrogen-bonded (5182 cm^{-1}) water. It is proved that the ratio of weakly hydrogen-bonded water is getting high in normal eggs by taking ratio (5282/5182 cm^{-1}) as shown in Figure 3(b). It is likely that life events occurring within the egg during development

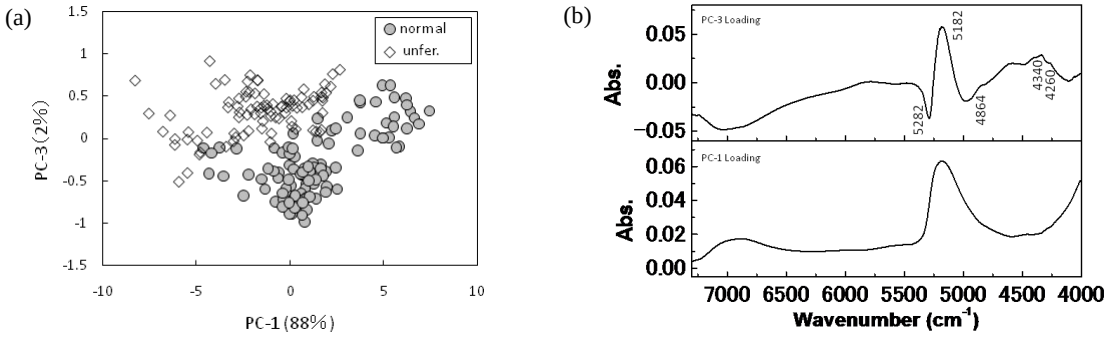

Figure 2: (a) Score plot of PC1 and PC3. (b) Loading plots of PC1 and PC3.

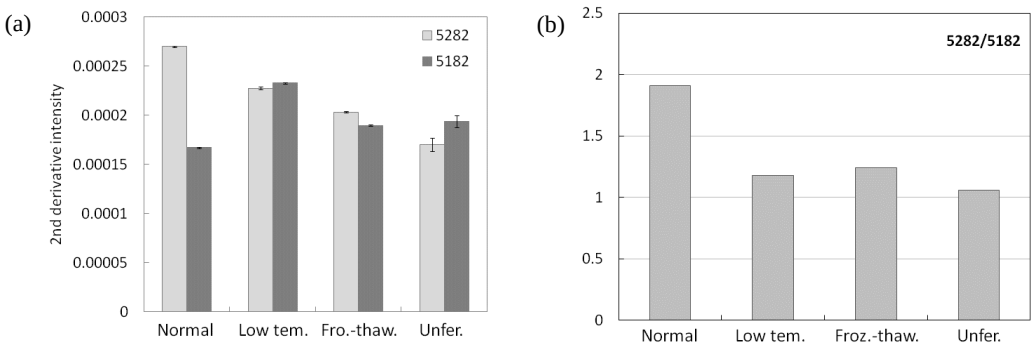

Figure 3: (a) The second derivative intensities at 5282 and 5182 cm^{-1}. (b) The intensity ratio of 5282/5182 cm^{-1}.

were shown to have an impact on the hydrogen bonding of water. Figure 4 shows NIR imaging of protein and water (strongly hydrogen-bonded water) obtained by plotting second derivative intensity. The differences of material variations and distributions can be visualized between normal and abnormal eggs.

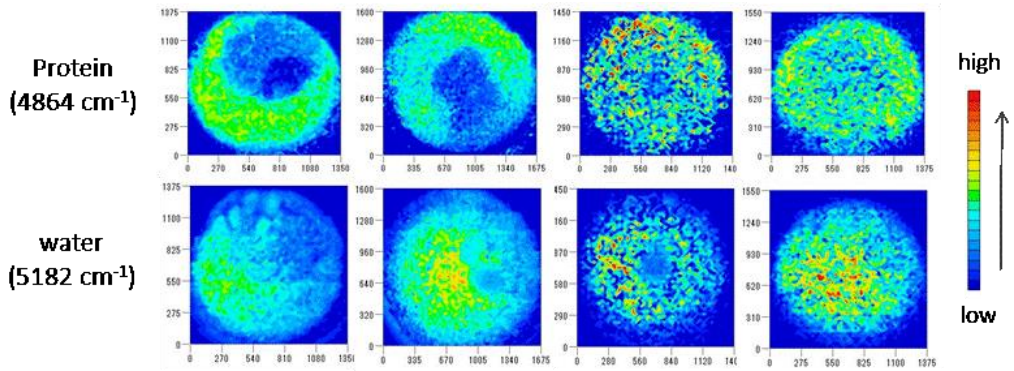

Figure 4: NIR imaging of protein and water (strongly hydrogen-bonded water).

Next, the differences of water structure depending on three parts (yolk, oil droplets, and embryo) were analyzed. It was confirmed that oil parts and embryo parts had high concentrations of strongly hydrogen-bonded and weakly hydrogen-bonded water, respectively. In order to describe the embryo parts more detail, PCA analysis was performed to the data set including point data of yolk and embryo of all days in 6000-7550 cm^{-1} region with focusing the variation of water structure.

The data set of yolk and embryo is classified into two groups by PC1 component. The loading plot of PC1 exhibited water band around 7000 cm^{-1} and the ratio of water bands (6850/7017 cm^{-1}) corresponded to weakly hydrogen-bonded water. That is, yolk and embryo parts can be distinguished by water structure, and embryo parts have relatively high percentage of weakly hydrogen-bonded water. PC1 loading was projected on imaging data to get scalar values as score numbers. The scores were plotted to obtain images with regards to the water structure variations were obtained as shown in Figure 5. The pixels with higher ratio of weakly-hydrogen bonded water were highlighted. The detailed structure of embryo, and membranes of egg and oil droplets were emerged. About the cell membrane structure, lipid bilayer are composed of two parts showing hydrophilic and hydrophobic properties. Hydrophobic tails consisting of fatty acids are not hydrated because they are not electrically polarized. That is, water does not interact with the hydrophobic part by hydrogen bonding. Therefore, the ratios of weakly hydrogen-bonded water get high within the membrane structure.

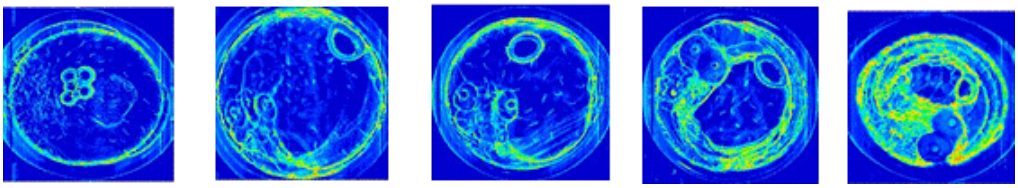

Figure 5: NIR images of water structural difference developed by using PCA result.