4P086

Sensing Cellular Metabolic States by Time Resolved Fluorescence of NADH

(RIES, Hokkaido Univ.[1]; Dep. of Appl. Chem., Univ. of Miyazaki[2]) ○Md. Serajul Islam[1], Takakazu Nakabayashi[1], Jin Matsumoto[2], Masahide Yasuda[2], Nobuhiro Ohta[1]

[Introduction] Probing cellular environment by monitoring autofluorescence has been widely used in biological research because it can be applied to living cells under native physiological condition. The coenzyme nicotinamide adenine dinucleotide (NADH) is one of the naturally occurring cellular autofluorescent species which play a key role in a number of physiological processes. Generally, in living cells, the NADH molecule exists in two forms: free and protein-bound. The change in lifetime of NADH fluorescence from the one of the free form to that of the bound form differs as much as 10 times. The relative amount of free and protein bound NADH and their lifetimes depend on the intracellular environments. In this presentation, we will show that the fluorescence lifetime of intracellular NADH can be employed to detect the change in the physiological states resulting from treatments such as (A) Cd treatment, (B) acetic acid treatment, (C) alteration in intracellular pH in yeast cells and in HeLa cells.

[Experimental] Measurements of fluorescence decay and time resolved emission spectra (TRES) were carried out using a single photon counting method. The second harmonic of the output from a femtosecond mode-locked Ti:Sapphire laser was used for excitation. The repetition rate of the laser pulse was selected to be 5.8 MHz with a pulse picker. Fluorescence from the sample was dispersed with a monochromator and detected with a microchannel-plate photomultiplier.

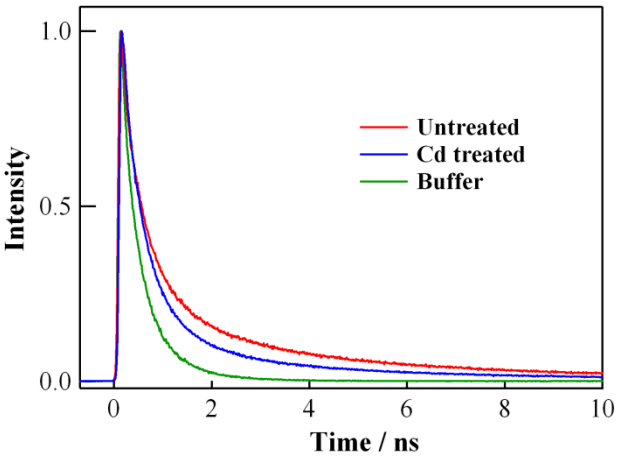

Fig. 1. Fluorescence decay profile of NADH in Cd treated yeast cells (blue line), untreated yeast cells (red line) and in buffer (green line). Excitation was a 370 nm laser pulse and the fluorescence was detected at 460 nm.

[Results and Discussion]

(A) Cd treatment: The heavy metal Cd has been reported to perturb the usual metabolic pathway of living cell. The toxicity of Cd has been investigated in yeast cells in that it causes apoptosis. Fig. 1 shows the fluorescence decay profiles of NADH in yeast cells and in buffer. It is clear that the decay of NADH in yeast cells becomes faster with Cd treatment. These decays in the cells were fitted with multiexponential polynomials. Fluorescence lifetime of free and protein bound NADH in untreated yeast cells, estimated from the fitted curve, were 0.3 ns and 1.6 ns respectively. In the Cd treated yeast cells,

these two lifetime components were estimated to be 0.3 and 1.3 ns respectively. That is, with Cd treatment, fluorescence lifetime of free NADH in yeast cells remain unchanged but that of the protein bound component decreases nearly 20%.

Fig. 2 shows the TRES of NADH in untreated yeast cells. The fluorescence spectrum at different time interval shows peak at different wavelengths. This is because the earlier time interval is dominated by the fluorescence from the free NADH and latter intervals are dominated by the protein bound NADH. Therefore, the protein bound NADH exhibits a blue shifted spectrum compared to the free one. In Fig. 3, the fluorescence spectrum in 2.0-5.0 ns time range, obtained from the TRES of NADH in Cd treated yeast cells, has been shown together with that in untreated cells. It is clear that the fluorescence spectrum of protein bound NADH in Cd treated yeast cells is shifted to a longer wavelength compared to that in the untreated cell. This shift is due to weakening of the bond between NADH and protein by the Cd treatment.

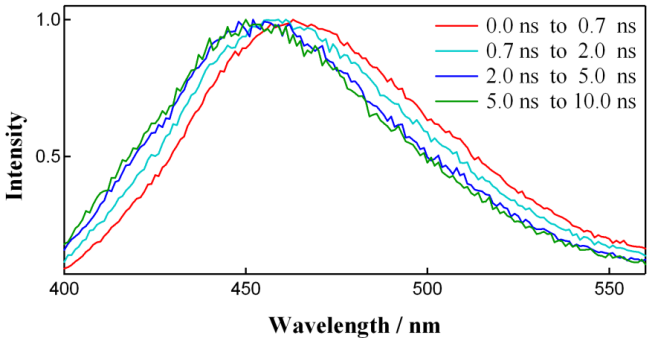

Fig. 2. TRES of NADH in yeast cells. The red, cyan, blue and green lines are 0.0-0.7, 0.7-2.0, 2.0-5.0 and 5.0-10.0 ns time interval, respectively.

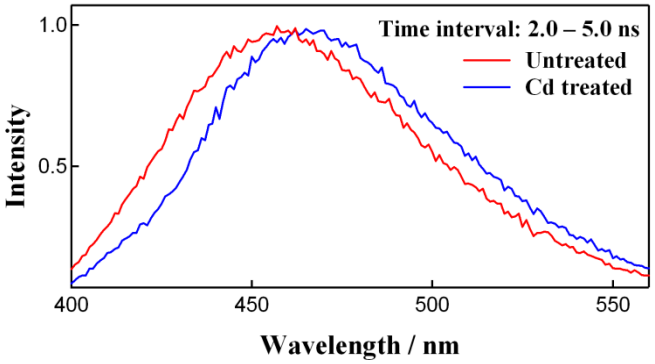

Fig. 3. The red and blue lines are the 2-5 ns components of the TRES of NADH in untreated and Cd treated yeast cells, respectively.

(B) Acetic acid treatment: Acetic acid has been reported to induce apoptosis by generating reactive oxygen species. Acetic acid is produced when metabolic pathway is deviated from the usual mitochondria mediated oxidative phosphorylation towards cytosolic glycolysis. The fluorescence decay and TRES of NADH in acetic acid treated yeast cells were also measured. In contrary to the Cd treatment, the fluorescence decay of NADH becomes slower with acetic acid treatment. No change in the TRES has been observed in acetic acid treated yeast cells.

(C) Alteration in intracellular pH: Intracellular pH level is intimately linked with the physiological processes occurring inside the cell. To correlate the fluorescence lifetime of NADH with intracellular pH, we measured fluorescence decay of NADH in HeLa cell at different intracellular pH[1]. Our experimental result exhibits that the fluorescence lifetime of NADH in HeLa cell increases with the decrease of intracellular pH. That is, the fluorescence lifetime of NADH in living cells can be employed for noninvasive detection of the intracellular pH.

[1] S. Ogikubo, T. Nakabayashi, T. Adachi, M. S. Islam, T. Yoshizawa, M. Kinjo, N. Ohta, *J. Phys. Chem. B*, In press.