Cellular flavoprotein fluorescence imaging for the analysis of mitochondrial function

Iyuki Namekata[1], Seri Kaeriyama[1], Reina Ikaga[2], Hikaru Tanaka[1], and Naoko Iida-Tanaka[3]

[1]Department of Pharmacology, Toho University Faculty of Pharmaceutical Sciences
2-2-1 Miyama, Funabashi-shi, Chiba, Japan 274-8510
[2]Institute of Human Culture Studies, Otsuma Women's University
12 Sanban-cho, Chiyoda-ku, Tokyo, Japan 102-8357
[3]Department of Food Science, Faculty of Home Economics, Otsuma Women's University
12 Sanban-cho, Chiyoda-ku, Tokyo, Japan 102-8357

Key words：Mitochondria, Fluorescence Imaging, Lifestyle Diseases

Abstract

Mitochondrial function is the key factor in the regulation of energy metabolism and is closely related to the generation and prevention of lifestyle-related diseases. We established a cellular fluorescence imaging method for the evaluation of mitochondrial function. Confocal microscopy revealed that the flavoprotein fluorescence was of mitochondrial origin. The flavoprotein fluorescence intensity was increased under elevated electron transport activity, while decreased under reduced electron transport activity. Activation of the mitochondrial ATP-sensitive K^+ channel induced an increase in flavoprotein fluorescence. The present imaging method would be useful for further studies on the regulation of mitochondrial function and its nutritional significance.

1. Introduction

Mitochondrial function is the key factor in the regulation of energy metabolism and is closely related to the generation and prevention of lifestyle-related diseases[1-6]. Skeletal and cardiac muscles, major energy consumers in the human body, largely rely on the mitochondrial oxidative phosphorylation as a source of cellular ATP. Loss of mitochondrial function plays a significant role in the pathophysiology of ischemic diseases including angina pectoris and myocardial infarction[7,8]. For the studies of the molecules involved in mitochondrial function, isolated mitochondria or mitochondrial homogenates has been widely used[9]. For the analysis of mitochondrial regulation, however, observation of mitochondrial function in the intact cell is highly desired. Thus, in the present study, we intended to establish a cellular fluorescence imaging method for the evaluation of mitochondrial function. We focused on cellular flavoprotein fluorescence as an index of mitochondrial respiration[10,11]. We visualized the intracellular localization of flavoprotein fluorescence and pharmacologically analyzed its correlation with mitochondrial electron transport activity and intracellular ATP status.

2. Methods

Guinea-pig ventricular cardiomyocytes were obtained by Langendorff perfusion of the heart with collagenase solution as previously described[8]. Mouse fibroblast-derived 3T3-L1 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose (GIBCO) under humidified 5% CO_2 atmosphere. On observation, cells were placed in a chamber on the stage of a confocal microscope (LSM510, Carl Zeiss) or an epifluorescent microscope (IX70, Olympus) and maintained at 37°C. Two dimensional images of cellular flavoprotein

fluorescence were obtained under excitation at 488nm. The time course of the whole cell fluorescence intensity was calculated with Aquacosmos software (Hamamatsu Photonics). Tetramethylrhodamine ethylester (TMRE) fluorescence was imaged under excitation at 543nm by confocal microscopy as in our previous studies[8,12].

3. Results

In isoltated guinea-pig cardiomyocytes, flavoprotein fluorescence was observed throughout the cell except for the nuclei in a streaky fashion (Fig. 1A). Staining of the cell with TMRE, a fluorescent mitochondrial marker, resulted in a similar fluorescence pattern (Fig. 1B). Merging of the two images revealed that they were in good correlation (Fig. 1C).

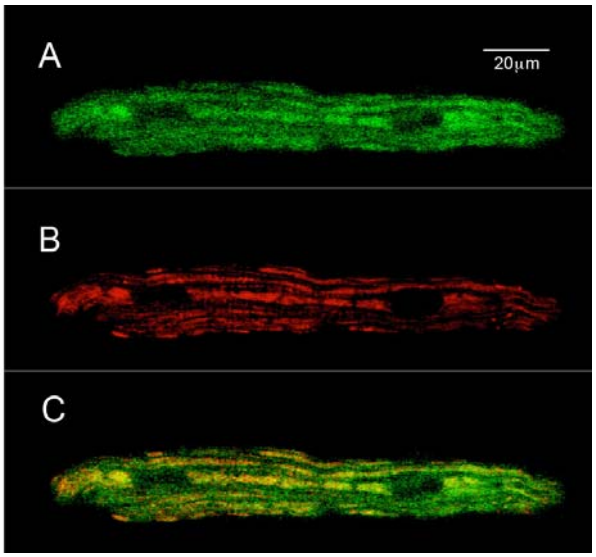

Figure 1. Flavoprotein fluorescence in a cardiomyocyte. A: cellular autofluorescense under excitation at 488nm. B: TMRE. C: Merge

Application of a mitochondrial uncoupler, 2,4-dinitrophenol (DNP; 100μM), resulted in an increase in fluorescence (Fig. 2A), while reduction of fluorescence was observed after further application of potassium cyanide (KCN; 4mM), an inhibitor of the mitochondrial electron transfer (Fig. 2A). Also in cultured 3T3-L1 cells, the cytoplasmic fluorescence was increased by a mitochondrial uncoupler, trifluorocarbonylcyanide phenylhydrazone (FCCP; 5μM; Fig. 2Ba) and decreased by sodium cyanide (1mM; Fig. 2Bb)

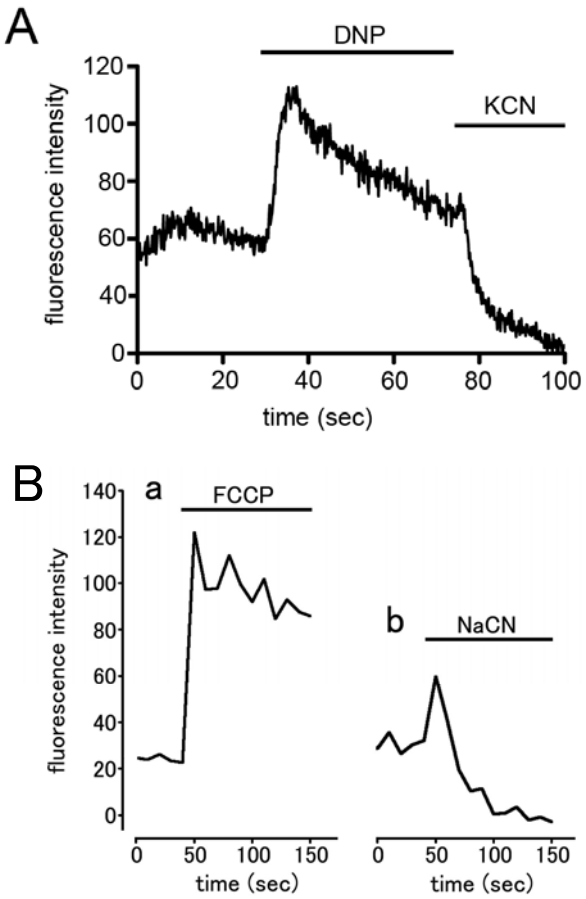

Figure 2. Effect of electron transport activity on flavoprotein fluorescence in a cardiomyocyte (A) and 3T3-L1 cell (B)

In isolated cardiomyocytes, application of diazoxide (100μM), an opener of the mitochondrial ATP-sensitive K^+-channel, induced an increase in flavoprotein fluorescence (Fig. 3). When applied in the presence of 5-hydroxydecanoate (5-HD; 500μM), an inhibitor of the mitochondrial ATP-sensitive K^+-channel, the diazoxide-induced increase in flavoprotein fluorescence was markedly reduced.

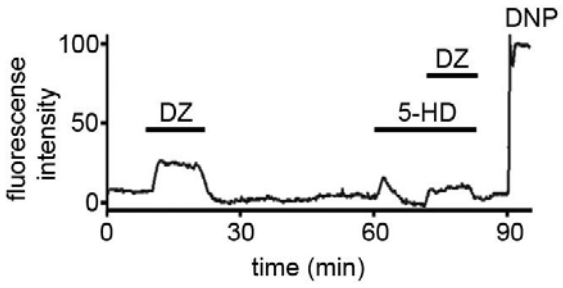

Figure 3: Effect of cellular energy status on flavoprotein fluorescence intensity

4. Discussion

Various cell types show autofluorescense in the wavelength range of 500 to 600nm due to flavoproteins present in the mitochondria[10,11]. The chromophore of flavoproteins is the flavin-adenine dinucleotide (FAD) whose fluorescence intensity is sensitive to its oxidation-reduction status; the oxidized form, FAD, is fluorescent, but the reduced form, $FADH_2$, is not. In the present study, we obtained images of flavoprotein fluorescence in intact cells to demonstrate its value for the investigation of mitochondrial function. The cellular distribution of flavoprotein fluorescence correlated well with that of TMRE indicating that the flavoprotein fluorescence originates mainly in the mitochondria (Fig. 1).

We next examined the relationship of flavoprotein fluorescence intensity and mitochondrial electron transport activity using pharmacological interventions. The mitochondrial uncouplers, DNP and FCCP, abolish the proton concentration gradient across the mitochondrial inner membrane and accelerate the electron transport. Conversely, cyanide inhibits the electron transport by inhibiting the complex IV. In the present study, application of mitochondrial uncouplers markedly increased, while cyanide reduced cellular flavoprotein fluorescence (Fig. 2). This indicates that increases and decreases in the flavoprotein fluorescence of intact cells reflects increases and decreases in the mitochondrial electron transport activity, respectively. Our present observations in cardiomyocytes (Fig. 2A) agree with those of earlier studies[13]. Similar phenomenon was observed in 3T3-L1 cells (Fig. 2B) and has also been reported in neurons[14]. Thus, flavoprotein fluorescence appears to be a useful indicator of electron transport activity in various cell types.

We further examined whether flavoprotein fluorescence is influenced by cellular energy status. In general, mitochondrial electron transport is accelerated under reduced cellular ATP concentration. One of the mechanisms involved is the mitochondrial ATP-sensitive K^+ channel present on the inner membrane. A decline in ATP concentration allows opening of the channel, resulting in depolarization of the mitochondrial inner membrane which is considered to accelerate the electron transport flow[15]. Diazoxide, which mimics ATP deficiency by opening the mitochondrial ATP-sensitive K^+ channel, produced an increase in flavoprotein fluorescence; this phenomenon was not observed in the presence of 5-hydroxydecanoate, an inhibitor of the mitochondrial ATP-sensitive K^+ channel (Fig. 3). These results indicate that the flavoprotein fluorescence is sensitive to ATP concentration and thus reflects cellular energy status. Recent studies suggest that flavoprotein fluorescence is also related to intracellular Ca^{2+} dynamics[16]. Thus, flavoprotein fluorescence measurement provides a means to study the factors involved in the regulation of mitochondrial function in intact cells under physiological and various pathophysiological conditions. Its contribution to nutritional science is anticipated.

5. References

[1] Duchen MR. Mitochondria in health and disease: perspectives on a new mitochondrial biology. Mol Aspects Med. 2004, 25(4), p.365-451
[2] Duchen MR et al. Roles of mitochondria in human disease. Essays Biochem. 2010, 47, p.115-137
[3] James AM et al. Mitochondrial oxidative stress and the metabolic syndrome. Trends Endocrinol Metab. 2012, 23(9), p.429-434
[4] Kusminski CM et al. Mitochondrial dysfunction in white adipose tissue. Trends Endocrinol Metab. 2012, 23(9), p.435-443
[5] Hoeks J et al. Muscle mitochondria and insulin resistance: a human perspective. Trends Endocrinol Metab. 2012, 23(9), p.444-450

[6] Pintus F et al. Nutrient availability links mitochondria, apoptosis, and obesity. AGING. 2012, 4(11), p.734-741
[7] Stanley WC et al. Energy metabolism in the normal and failing heart: potential for therapeutic interventions. Heart Fail Rev. 2002, 7(2), p.115-130
[8] Namekata I et al. Reduction by SEA0400 of myocardial ischemia-induced cytoplasmic and mitochondrial Ca^{2+} overload. Eur J Pharmacol. 2006, 543, p.108-115
[9] Perry CG et al. Methods for assessing mitochondrial function in diabetes. Diabetes. 2013, 62(4), p.1041-1053
[10] Benson RC et al. Cellular autofluorescense: is it due to flavins? J Histochem Cytochem. 1979, 27(1), p.44-48
[11] Scholz R et al. Flavin and pyridine nucleotide oxidation-reduction changes in perfused rat liver. J Biol Chem. 1969, 244(9), p.2317-2324
[12] Iida-Tanaka N et al. Membrane-labeled MDCK cells and confocal microscopy for the analyses of cellular volume and morphology. Biol Pharm Bull. 2008, 31(4), p.731-734
[13] Romashko DN et al. Subcellular metabolic transients and mitochondrial redox waves in heart cells. Proc Natl Acad Sci USA. 1998, 95, p.1618-1623
[14] Shuttleworth CW. Use of NAD(P)H and flavoprotein autofluorescense transients to probe neuron and astrocyte responses to synaptic activation. Neurochem Int. 2010, 56(3), p.379-386
[15] Liu Y et al. mitochondrial ATP-sensitive potassium channels: novel effectors of cardioprotection? Circulation. 1998, 97, p.2463-2469
[16] Iino M et al. Spatiotemporal dynamics of Ca^{2+} signaling and its physiological roles. Proc Jpn Acad Ser B. 2010, 86, p.244-256
(Received October 25, 2013; accepted November 5, 2013)

細胞フラボプロテイン蛍光イメージングによるミトコンドリア機能解析

行方 衣由紀，帰山 世理，伊香賀 玲奈，田中 光，田中 直子

キーワード：ミトコンドリア，蛍光イメージング，生活習慣病

抄録

ミトコンドリア機能はエネルギー代謝の制御に中心的役割を果たしており，生活習慣病の発生・予防とも深く関連している．我々は細胞のフラボプロテイン蛍光イメージングによりミトコンドリア機能を計測する方法を確立した．共焦点顕微鏡法により，フラボプロテイン蛍光がミトコンドリアに由来することを確認した．フラボプロテイン蛍光は電子伝達が亢進した状態で増大，抑制された状態で減弱することを観測した．またミトコンドリア内膜のATP感受性カリウムチャネルの開口による蛍光の増大を観測した．本研究で確立した蛍光イメージングによる解析法は，ミトコンドリア機能の制御機構およびその栄養学的意義の解明に有用である．

行方　衣由紀（なめかた　いゆき）
現職：東邦大学薬学部薬物学教室講師

東邦大学大学院薬学研究科博士後期課程修了．
専門は循環薬理学．細胞内事象の蛍光イメージング法と薬理学的・電気生理学的手法を組み合わせた総合的検討により，心臓機能を包括的に理解することを目指して研究を行っている．