

## A NEW IMAGING METHOD OF GLUCOSE TRANSPORT IN LIVE CELLS

Katsuya Yamada<sup>1)</sup>, Toshihiro Yamamoto<sup>2)</sup>, Seiji Watanabe<sup>3)</sup>, Yuji Nishiuchi<sup>2)</sup>,  
Tadashi Teshima<sup>2)</sup>, Hideaki Matsuoka<sup>4)</sup> and Sechiko Suga<sup>5)</sup>

**Abstract** Glucose transport activity in mammalian cells has been monitored by radiolabeled tracers such as [<sup>14</sup>C] 2-deoxy-D-glucose. However, due to their limited spatial and temporal resolution, measuring glucose uptake in single, living cells was difficult. We have developed a fluorescent D-glucose derivative, 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG), that allows a more sensitive measurement of glucose uptake in live cells. Indeed, the uptake of 2-NBDG takes place through glucose transporters (GLUTs) in a concentration-, time-, and temperature-dependent manner. Kinetic analysis revealed that apparent  $K_m$  values for the uptake were similar to those reported by D-glucose and the nonmetabolizable glucose analogue, 3-O-methyl-D-glucose, found in pancreatic islet cells. This method can be combined with  $Ca^{2+}$  imaging and subsequent immunocytochemical identification of cells. So far, 2-NBDG has been used for monitoring glucose uptake into a wide variety of mammalian cells including astrocytes and neurons. However, it has been pointed out that 2-NBDG method requires very accurate procedures, since the fluorescence intensity is an arbitrary measure. In addition, temporal changes in health of cells in question should be verified throughout the experiment by an independent way such as using patch clamp. To overcome the difficulty, we synthesized transporter-unrecognizable glucose analogues as control for 2-NBDG uptake. These include 2-[N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-L-glucose [2-NBDLG], the antipode of 2-NBDG. A combined use of transporter-recognizable (D-isomer) and unrecognizable (L-isomer) fluorescent analogues allows us to investigate not only net stereospecific transport of glucose in live cells but also ligand-transporter interactions and non-transporter-mediated glucose movement.

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D-glucose is one of the most important energy sources for various organisms from *E. coli* to mammals to survive. Recent advances in molecular techniques have unveiled growing numbers of glucose transporters such as GLUTs that may be located in particular sites of the plasma membrane<sup>1)</sup>. In addition, translocation of some transporters in response to insulin stimulation has been documented, raising a question what precise roles of these divergent glucose transporters are<sup>2)</sup>. Historically, glucose transport activity has been monitored by radiolabeled tracers such as [<sup>14</sup>C] 2-deoxy-D-glucose<sup>3)</sup>. However, they are not suitable for

detecting glucose uptake at the single-cell level in real time due to their poor spatial and temporal resolution.

In 1996, we developed a fluorescent D-glucose derivative, 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino]-2-deoxy-D-glucose [2-NBDG] (Fig. 1) to see viability of *E. coli*<sup>4)</sup>. Then we proved that 2-NBDG is incorporated into mammalian cells through glucose transporters in a time, concentration, and temperature-dependent manner with  $K_m$  values similar to those reported by radiolabelled glucose tracers<sup>5)</sup>.

So far 2-NBDG has been successfully applied to various organisms by different groups<sup>6)</sup>. Of

<sup>1)</sup> Dept. Physiol., Hirosaki Univ. Grad. Sch. Med., Hirosaki, 036-8562 Aomori, Japan.

<sup>2)</sup> SAITO Res. Center, Peptide Institute, Inc., Ibaraki, Osaka 567-0085, Japan.

<sup>3)</sup> Department of Anatomical Science, Hirosaki Univ. Grad. Sch. Med., Hirosaki, Japan.

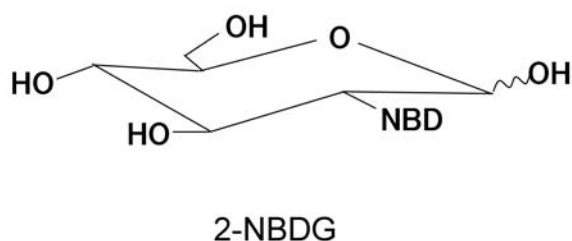
<sup>4)</sup> Department of Biotechnology and Life Science

Faculty of Technology, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan.

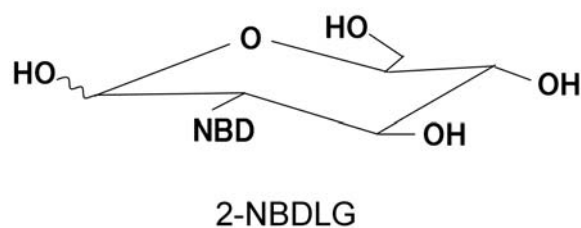
<sup>5)</sup> Center Res. & Edu. Lifelong Learning, Hirosaki Univ., Hirosaki, Japan.

e-mail: kyamada@cc.hirosaki-u.ac.jp

fax: +81-172-39-5009



**Fig. 1** Chemical structure of 2-NBDG. See text for the chromophore, NBD.



**Fig. 2** Chemical structure of 2-NBDLG.

particular interest is its application to the brain<sup>7,8</sup>, which utilizes glucose as a sole energy source, and to malignant tumor cells<sup>9</sup>. However, care should be taken in that the fluorescence intensity is an arbitrary measure and that previous 2-NBDG methods had no control fluorescent substrate. This is important particularly when applied to tissues consisting of heterogeneous cells expressing GLUTs with low  $K_m$  values and showing divergent activity as seen typically in the brain<sup>6</sup>.

It is known that mammalian cells predominantly incorporate D-isomer of glucose<sup>10</sup>. Thus, we synthesized an enantiomer of 2-NBDG (named as 2-NBDLG (Fig. 2)) as a control substrate to solve problems encountered when 2-NBDG alone was applied<sup>11</sup>. Measurement of the difference in the fluorescence derived from 2-NBDG and 2-NBDLG would provide critical information on the net stereospecific uptake of D-glucose into single, living cells, setting it apart from other factors such as non-specific uptake and/or transporter-unrelated binding to the cellular surface that can be serious problems in some application<sup>12</sup>.

L-Glucosamine was synthesized from L-mannose in 10 steps<sup>11</sup>. The <sup>1</sup>H-NMR data of synthetic L-glucosamine thus obtained, was completely identical with that of commercially available D-glucosamine. On the other hand, optical purity of L-glucosamine was confirmed by the comparison of specific rotation with that of D-glucosamine. Optically pure L-glucosamine thus obtained was coupled with 4-fluoro-

7-nitrobenz-2-oxa-1,3-diazole (NBD-F) to give 2-NBDLG in 76% yield.

So far, we have applied 2-NBDG and 2-NBDLG to various cells and tissues including brain cells such as glia and spontaneously active neurons. Many previously unaware phenomena have been noticed by using 2-NBDLG as a control. Some of results were presented elsewhere in abstract form<sup>13</sup>. Use of confocal microscopy is helpful for rough estimation of transporter-unrelated binding of 2-NBDG to the cellular surface. Non specific uptake of the glucose derivatives through pathways other than stereo-selective glucose transporters can be detected by using 2-NBDLG as a control. Especially, changes in the membrane integration of delicate nerve cells during measurement could be detected by using L-form isomers. On the other hand, detection of small and slow processes of 2-NBDG uptake through such as GLUT3 and GLUT1 in brain cells requires a relatively strong light for excitation, which might produce light-induced damage to the cells of interest. Recent development of high S/N photomultipliers would greatly reduce the damage and makes it possible to use more frequent exposure to light required for a real-time measurement of dynamic uptake processes.

Use of 2-NBDG has brought exciting implications including such as metabolic wave<sup>7,8</sup> and intercellular transport of D-glucose and/or its phosphorylated form through gap junction<sup>14</sup>. By using of transporter- recognizable (D-isomer)

and unrecognizable (L-isomer) fluorescent analogues combined with modern live-cell imaging techniques should provide valuable information on dynamism of glucose transport.

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