INVESTIGATION OF ENZYME REACTION IN EXTENDED-NANO SPACE MIMICKING CELLULAR ENVIRONMENTS

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ABSTRACT

We have succeeded in investigating enzyme reaction in extended-nano (10-1000 nm) space modified by phospholipid bilayers which mimics an intercellular structure (from several tens to several hundreds nanometer-spacing). Previously, enzyme reaction in intercellular space was difficult to detect both *in vivo* and *in vitro*. However, this analytical model enables to investigate enzyme reaction in intercellular space, and will be powerful tools for study on cell functions close to cell membranes.

KEYWORD

Extended-nano space, enzyme reaction rate, lipid bilayers modification

INTRODUCTION

Many researchers have made a study on cell functions in cells space of 10-1000 nm, and in such space it is suggested that specific solution properties such as higher viscosity and higher proton mobility [1]. However, direct detection of those in vivo is challenging. Therefore, it is required to make a tool for investigating cells space *in vitro*, but the tool is unrealized.

So far, the biomimetic extended-nano space of 10-1000 nm, which was made on fused silica and modified by phospholipid bilayer, was developed. In this space, higher proton mobility was observed than in bulk. However, how the specific property near cell membrane affects cells function has not been investigated yet.

In particular, we focused on enzyme reaction, which plays an important role in cells function. In this study, the effect of higher proton mobility on enzyme reaction is investigated. We expect the structured water molecules help protonation to substrate (Figure 1).

Therefore, the objective is revealing relationship between solution property and cells function by the biomimetic extended-nano space. The issue is selecting reaction system and designing micro-nano chip for reaction rate measurement.

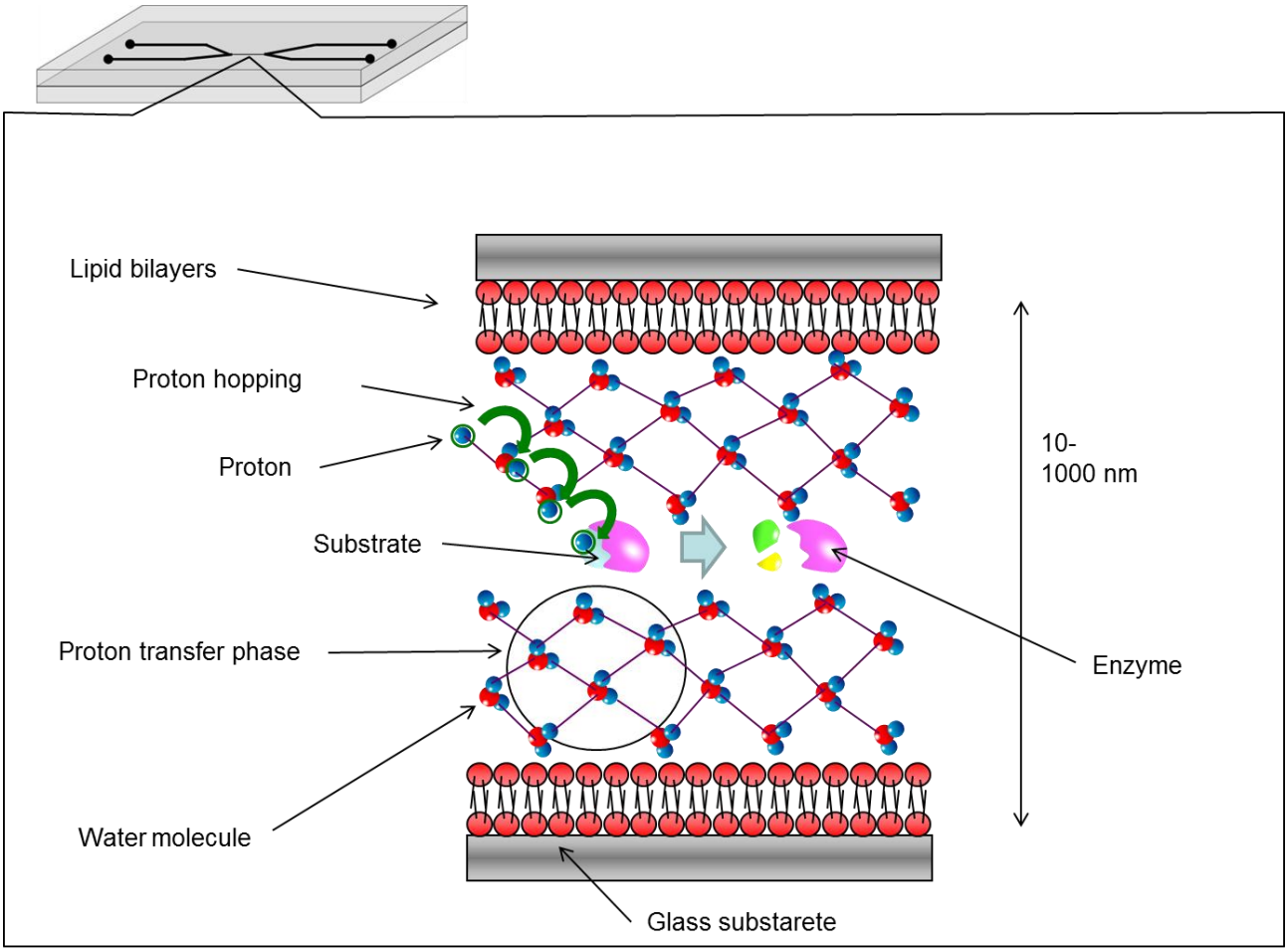

Figure 1.Concept of this study

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EXPERIMENTAL

The extended-nano channels were fabricated on a synthetic quartz glass by electron beam lithography and plasma etching. In this study, plate-type extended-nano channel (width 100 µm, depth 300 nm, length 400 µm) and square-type extended-nano channel (width 600 nm, depth 300 nm, length 400 µm) were used. Then micro channels and holes for sample introduction were fabricated on the chip. The chip was thermally bonded with an upper plate at the temperature of 1080 ℃.

As a suitable enzymatic reaction, we selected a hydrolysis reaction in which nonfluorescent TG-β-gal was hydrolyzed to TG and β-galactose by β-gal acting as catalyst, as shown in Figure 2. The observed reaction product, which was TG, exhibited strong fluorescence at around 510 nm. In this reaction, protonation to Tokyogreen-β-galactoside might be affected by increase in proton mobility in extended-nano space

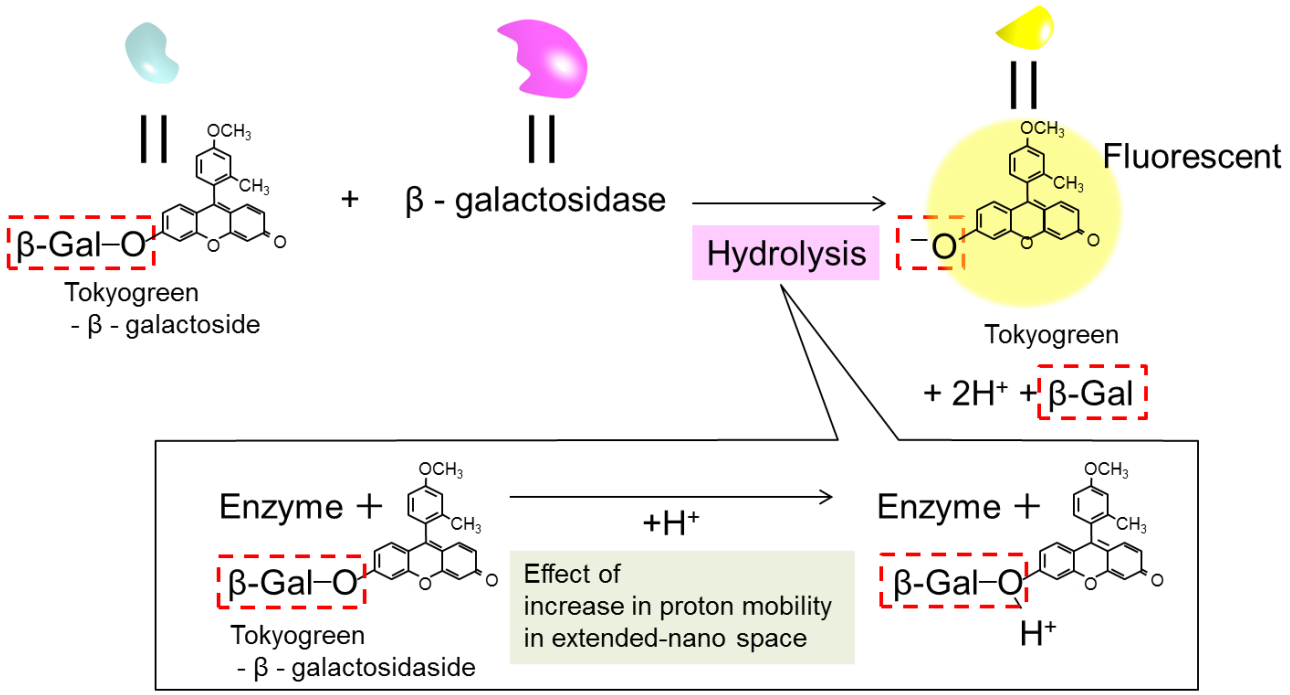

Figure 2.Hydrolysis of Tokyogreen-β-galactoside

Lipid bilayers were modified by vesicle fusion method. As lipids, dioleoylphosphatidylcholine (DOPC) including Texas-Red dihexadecanoyl-sn-grycero-3-phosphoethanolamine (DHPE) 1% was used. Vesicle solution was introduced in extended-nano channel, and then buffer solution was introduced for washing.

Enzyme solution and substrate solution was mixed in micro channel, and was injected into lipid modified square-type extended-nano channel in 200 kPa with pressure controllers (Figure 3). In this chip design and pressure, injection time was estimated at 12 seconds, and this is much shorter than reaction time: 30 minutes. Therefore, initial rate V_0 was determined in this system. After sufficiently introducing mixed solution of substrate and enzyme into extended-nano channel, pressure was turned off and flow was stopped. Fluorescent intensity to reaction time was measured at enzyme concentration of 10 µM and substrate concentration [S] of 5µM, 10µM, and 20µM. Similarly in plate-type extended-nano channel, micro channel, and bulk, fluorescent intensity to reaction time was measured.

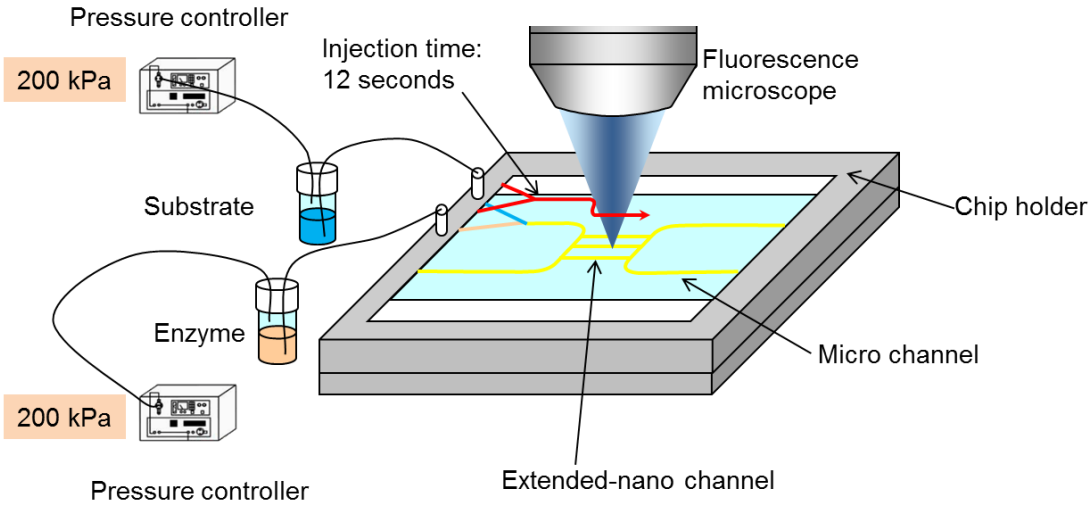

Figure 3.Setup

RESULTS AND DISCUSSION

Figure 4 shows relation between reaction time and fluorescent intensity in lipid modified square-type extended-nano channel. In case of introduction of enzyme and substrate into extended-nano channel, fluorescent intensity increased with increasing reaction time. However, in case of introduction of blank solution, fluorescent intensity didn't increase. Therefore, enzyme reaction in lipids modified square-type extended-nano channel was monitored successfully by fluorescence. It was the same in case of the other channels. From the slope of increase in fluorescent intensity, V_0 was obtained. Lineweaver–Burk plot of $1/V_0$ vs. $1/[S]$ was used to determine the kinetic parameters of enzyme reaction. Maximum reaction rate V_{max} was obtained from the intercept, and Michaelis constant K_m was obtained from the intercept and the slope.

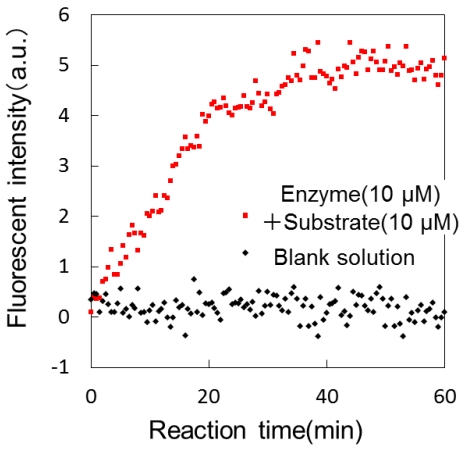

Figure 4.Relation between reaction time and fluorescent intensity in lipid modified square-type extended-nano channel

Table 1.K_m and V_{max} in each channel

	K_m	V_{max} (nM/S)
phospholipid bilayers / Square type: 600 nm × 300 nm	206	208
Square type: 600 nm × 300 nm	121	109
Plate type: 100 μm × 300 nm	81	56
Plate type: 100 μm × 300 nm	78	52
Micro channel (unmodified) (width 100 μm depth 6 μm)	72	45
Bulk	69	41

Table 1 shows K_m and V_{max} in each channel. V_{max} in square-type extended-nano channel is higher than that in plate-type extended-nano channel. Increase in V_{max} means increase in hydrolysis including protonation. And also this result corresponds to behavior of proton mobility. The reaction rate in lipid modified square-type extended-nano channel is higher than that in unmodified square-type extended-nano channel. Proton mobility in lipids modified extended-nano channel is also higher than that in unmodified extended-nano channel at 300 nm channel size. Therefore, proton of solvent might get involved in hydrolysis.

V_{max} of lipids modified square-type extended-nano channel is 5.1 times higher that of bulk. Converted to temperature using Arrhenius equation, this corresponds to increase of 27 ℃. This shows enzyme reaction near cell membranes is at higher velocity and more efficiently than expected. The tool which we developed enabled to imitate biological reaction near cell membranes in vitro for the first time.

CONCLUSION

Enzyme reaction rate in extended-nano space was determined successfully by micro-nano chip that we designed, and dependence of enzyme reaction with space size is investigated. Increase in enzyme reaction rate in intercellular space is verified *in vitro*. Therefore, it is demonstrated that *in vitro* model of intercellular space we developed is very useful.

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REFERENCES

1. T. Tsukahara, A. Hibara, et al., Angew.Chem.,Int.Ed., 46, 1180-1183(2007)

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